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UNIVERSITY OF ALBERTA

The Pharmacokinetics and Pharmacodynamics of Captopril in Infants with Congestive Heart Failure and in Piglets

by

Conrad Michael Pereira

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF

Doctor of Philosophy

IN

Pharmaceutical Sciences (Pharmacokinetics)

Faculty of Pharmacy and Pharmaceutical Sciences

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SPRING, 1992

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Dedication

For my Parents, Joy, Olga, Daphne and Charles.

Abstract

The angiotensin converting enzyme inhibitor, captopril, is presently being used, both in children and adults, for the treatment of hypertension and congestive heart failure (CHF). The use of captopril, specially in infants, has been empirical because pharmacokinetic information on captopril for this population has been unavailable. Although some data are available in adults, no clear kinetic-dynamic relationship has been defined for captopril. The objectives of this project were to determine standard pharmacokinetic parameters for captopril in infants with CHF and, using data from an animal model, to define a relationship between the pharmacokinetics and pharmacodynamics of captopril.

A simple, sensitive HPLC assay was developed for captopril and its disulphides in plasma. This method is suitable for use in infants. Previously available methods required too much (up to 15 ml) blood. Captopril is derivatized with N-(3-pyrenylmaleimide). The adduct is extracted and then assayed using reversed phase HPLC with fluorometric detection. Captopril disulphides are reduced with tributylphosphine before derivatization.

Captopril is unstable in solution and since it is usually administered to infants as a solution in tap water we studied the stability of captopril in this medium. The shelf-life of captopril in tap water, stored at 5°C, was estimated to be 26 days.

Standard pharmacokinetic parameters were determined for unchanged and total captopril in infants with CHF. It was found that these parameters were within the range reported for adults with CHF. Hemodynamic measurements made in these infants indicated that captopril had beneficial effects in these patients.

The piglet was investigated as an animal model in which to study the relationship between the pharmacokinetics and pharmacodynamics of captopril. The standard pharmacokinetic parameters for captopril in healthy anaesthetized piglets were found to be within the range reported for humans and the observed hemodynamic response was qualitatively similar to that in humans. Therefore the piglet was considered to be a viable animal model for our purpose. A parametric pharmacokinetic-pharmacodynamic model has been established to describe an effect versus concentration relationship for captopril and effect was found to increase linearly with concentration at the effect site(s).

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Table of Contents

PAGE

1. INTRODUCTION 1

1.1 History of Captopril 1

1.2 Congestive Heart Failure 1

1.3 Role of the Renin-Angiotensin System in CHF 2

1.4 Pharmacology of Captopril 4

1.4.1 Use of Captopril in CHF 5

1.5 Use of Captopril in Infants and Children . . 6

1.6 Side Effects of Captopril 8

1.7 Determination of Captopril in Biological Fluids 9

1.8 Pharmacokinetics of Captopril 12

1.8.1 Absorption 12

1.8.2 Distribution 14

1.8.3 Metabolism 15

1.8.4 Excretion 17

1.8.5 Summary of Pharmacokinetic Parameters 18

1.9 The Relationship between Pharmacokinetics and Pharmacodynamics 18

1.9.1 Pharmacodynamic Models 20

1.9.2 Pharmacokinetic-Pharmacodynamic Models 25

1.10 Relationship between the Pharmacokinetics and

Pharmacodynamics of Captopril 29
1.11 The Piglet as an Animal Model 32
1.12 Hypotheses 34
1.13 Objectives 35
1.14 Figures 36
1.15 References 38

2. Simplified Determination of Captopril in Plasma by High-Performance Liquid Chromatography 61
2.1 Introduction 61
2.2 Experimental 62
  2.2.1 Materials 62
  2.2.2 Instruments 62
  2.2.3 Procedures for the determination of captopril in neonatal plasma 64
    2.2.3.1 Unchanged captopril 64
    2.2.3.2 Total captopril (captopril and its mixed disulphides) . . . 65
  2.2.4 Calibration curves for unchanged and total captopril 65
  2.2.5 Patient study 66
  2.2.6 Validation study for an adult trial . 66
    2.2.6.1 Unchanged captopril 66
    2.2.6.2 Total captopril 68
2.3 Results and Discussion 69

2.4 Tables 78

2.5 Figures 82

2.6 References 91

3. THE STABILITY OF CAPTOPRIL IN TAP WATER 95

3.1 Introduction 95

3.2 Method 96

3.3 Results and Discussion 98

3.4 Conclusion 99

3.5 Table 100

3.6 Figures 101

3.7 References 103

4. THE PHARMACOKINETICS OF CAPTOPRIL IN INFANTS WITH CONGESTIVE HEART FAILURE 105

4.1 Introduction 105

4.2 Patients and Methods 106

4.3 Results 109

4.4 Discussion 111

4.5 Tables 116

4.6 Figures 119

4.7 References 122

5. PHARMACOKINETIC-PHARMACODYNAMIC MODELLING FOR CAPTOPRIL IN HEALTHY ANAESTHETIZED PIGLETS:

APPLICATION TO HUMANS 125
5.1 Introduction 125
5.2 Methods 128
5.3 Results 134
5.4 Discussion 137
5.5 Conclusion 142
5.6 Tables 143
5.7 Figures 150
5.8 References 160

6. GENERAL DISCUSSION AND CONCLUSIONS 166

List of Tables

PAGE

Table 2.1 Inter- and intra-day assay variability for unchanged captopril in quality control samples. 78

Table 2.2 Inter- and intra-day assay variability for total captopril in quality control samples. 79

Table 2.3 Compilation of calibration curves for total captopril. I=intercept, S=slope of the regression equation. 80

Table 2.4 Compilation of calibration curves for unchanged captopril. I=intercept, S=slope of the regression equation. 81

Table 3.1 Summary of major chemical parameters for tap water in the City of Edmonton in 1988 as reported by the Rossdale Water Treatment Laboratory in Edmonton. 100

Table 4.1 Patient clinical data and concurrent medication. 116

Table 4.2 Pharmacokinetic parameters for unchanged captopril after a 1 mg/kg po captopril dose to 10 infants. 117

Table 4.3 Pharmacokinetic parameters for total captopril after a 1 mg/kg po captopril dose to 10 infants. 118

Table 5.1 Haemodynamic parameters in a sham experiment with a healthy piglet. 143

Table 5.2 Hemodynamic parameters in a sham experiment with a piglet with induced myocardial infarction. 144

Table 5.3 Non-compartmental pharmacokinetic parameter estimates for unchanged captopril after a 0.2 mg/kg iv captopril dose to 5 piglets. . . . 145

Table 5.4 Compartmental pharmacokinetic parameter estimates for unchanged captopril after a 0.2 mg/kg iv captopril dose to 5 piglets (2-compartment open model with bolus input). . 146

Table 5.5 Pharmacokinetic-pharmacodynamic model parameter estimates for aortic pressure after a 0.2 mg/kg iv captopril dose to 5 piglets. 147

Table 5.6 Pharmacokinetic-pharmacodynamic model parameter estimates for heart rate after a 0.2 mg/kg iv captopril dose to 5 piglets. . 148

Table 5.7 Compartmental pharmacokinetic model and kinetic-dynamic model parameter estimates for literature data. 149

List of Figures

PAGE

Figure 1.1 Simplified representation of the role of the renin-angiotensin-aldosterone system and the kallikrein-kinin-prostaglandin systems in the maintenance of the congestive heart failure state (*Romankiewicz et al. 1983*). 36

Figure 1.2 Summary of biotransformation of captopril in blood and urine. Proposed metabolic pathways are indicated by the dotted arrows (*Migdalof et al. 1980*). 37

Figure 2.1 Chemical structures of captopril (1), internal standard (2), captopril disulphide (3), N-(3-pyrenylmaleimide) (4) and proposed chemical structures of captopril-maleimide (5) and internal standard-maleimide (6) derivatives. 82

Figure 2.2 Typical chromatograms for unchanged captopril in plasma samples, using a Partisil® ODS-3 C_{18} column. (A) blank; (B) spiked with 476 ng/ml captopril; (C) patient. Peaks: c = captopril, s = internal standard. 83

Figure 2.3 Typical chromatograms for total captopril in plasma samples, using a Partisil® ODS-3 C_{18} column. (A) blank; (B) spiked with 162 ng/ml unchanged captopril equivalent; (C) patient. Peaks: c = captopril, s = internal standard. 84

Figure 2.4 Representative chromatograms, obtained using a μ-Bondapak® C_{18} (10 μm) column, for plasma samples. (A) blank; (B) spiked with 760 ng/ml moricizine; (C) spiked with captopril, internal standard and 760 ng/ml moricizine. Peaks: c = captopril, s = internal standard. 85

Figure 2.5 Representative chromatograms, obtained using a μ-Bondapak® C_{18} (10 μm) column, for plasma samples taken from a patient. (A) captopril administered alone; (B) captopril and moricizine administered simultaneously, showing interference with captopril peak. Peaks: c = captopril, s = internal standard. 86

Figure 2.6 Representative chromatograms, obtained using a Zorbax® C_8 column, for plasma samples. (A) blank; (B) spiked with 600 ng/ml captopril. Peaks: c = captopril, s

= internal standard. 87

Figure 2.7 Representative chromatograms, obtained using a Zorbax® C_8 column, for plasma samples taken from a patient. (A) captopril administered alone; (B) captopril and moricizine administered simultaneously, showing interference peak separated from captopril. Peaks: c = captopril, s = internal standard, i = interference peak. 88

Figure 2.8 Calibration curves for unchanged (●) and total (■) captopril. 89

Figure 2.9 Plasma concentration versus time curves for unchanged (●) and total (■) captopril after a 1 mg/kg oral dose to an infant. 90

Figure 3.1 Log % captopril remaining in tap water versus time at 5°C (△), 25°C (▲), 50°C (□) and 75°C (■). (Values shown for % captopril remaining are means ± SEM of 5 tablets each, determined in triplicate.) 101

Figure 3.2 Arrhenius plot for captopril in tap water. (K values shown are means ± SEM determined from 5 tablets each.) 102

Figure 4.1 Plasma concentration versus time profiles for unchanged (□) and total (■) captopril after a 1 mg/kg po captopril dose to 10 infants (means ± SD shown) 119

Figure 4.2 Heart (A) and respiratory (B) rates and mean pulmonary (C) and systemic (D) arterial pressures measured in 10 infants, before and 1 h after a 1 mg/kg po dose of captopril (means ± SD shown) 120

Figure 4.3 Systemic (A) and pulmonary (B) vascular resistances and left ventricular diastolic dimension (C) and ejection time (D) measured in 10 infants, before and 1 h after a 1 mg/kg po dose of captopril (means ± SD shown) 121

Figure 5.1 Pressure and flow tracings from a typical experiment with a healthy anaesthetized piglet. PA, pulmonary artery; LV, left ventricle; AO, aorta; RA, right atrium; LA, left atrium. 150

Figure 5.2 Plasma concentration versus time profile for captopril after a 0.2 mg/kg iv captopril dose to 5 piglets (means ± SD shown) 151

Figure 5.3 Aortic pressure versus time profile for captopril after a 0.2 mg/kg iv captopril dose to 5 piglets (means ± SD shown). Solid line represents model prediction. . 152

Figure 5.4 Heart rate versus time profile for captopril after a 0.2 mg/kg iv captopril dose to 5 piglets (means ± SD shown). Solid line represents model prediction. . 153

Figure 5.5 Plasma renin activity versus time profile after oral (A) and 'sublingual' (B) administration and systolic blood pressure (C) after 'sublingual' administration of 25 mg of captopril to healthy subjects (*Al-Furaih et al. 1991)*. Solid lines represent model predictions. 154

Figure 5.6 Plasma renin activity (A) and systemic vascular resistance (B) versus time profiles after administration of 25 mg captopril orally to patients with CHF (*Shaw et al. 1985*). Solid lines represent model predictions. 155

Figure 5.7 Systemic vascular resistance (A), cardiac output (B) and mean blood pressure versus time profiles after administration of 25 mg captopril intravenously to patients with congestive heart failure (*Rademaker et al. 1986*). Solid lines represent model predictions. 156

Figure 5.8 Percent inhibition of plasma converting enzyme activity versus time profile after administration of 50 mg captopril orally to hypertensive subjects (*Giudicelli et al. 1987*). Solid line represents model prediction. 157

Figure 5.9 Mean blood pressure versus time profile after administration of 50 mg captopril orally to patients undergoing peritoneal dialysis (*Fujimura et al. 1986*). Solid line represents model prediction. 158

Figure 5.10 Maximum change percent versus time: (●) aortic pressure in piglets after captopril 0.2 mg/kg iv, (□) mean blood pressure in patients undergoing peritoneal dialysis, after 50 mg captopril orally (*Fujimura et al. 1986*), (▿) mean blood pressure in patients with CHF after 25 mg captopril iv (*Rademaker et al. 1986*), (■) systolic blood pressure in normal subjects after administration of 25 mg captopril orally (*Al-Furaih et al. 1991*). 159

List of symbols and Abbreviations

AI	angiotensin I
AII	angiotensin II
ACE	angiotensin-converting enzyme
α	distribution rate constant
Ao	aorta
AoP	aortic pressure
AUC	area under the concentration versus time curve
β	elimination rate constant
BP	blood pressure
°C	degrees Centigrade
C_e	effect site concentration
CHF	congestive heart failure
Cl	clearance
Cl_{TB}	total body clearance
Cl_o	oral clearance
C	drug concentration
C_{max}	peak drug concentration in plasma after an oral dose
CO	cardiac output
CV	coefficient of variation
D	dose
dP/dt	rate of change of pressure
E	effect

E_0	baseline effect
EC_{50}	C producing 50 % of E_{max}
EDTA	disodium ethylenediamine tetraacetate
E_{max}	maximum effect attributable to drug
F	bioavailability
g	acceleration due to gravity (9.81 m $second^{-2}$)
GC-MS	gas chromatography-mass spectrometry
h	hour(s)
HPLC	high performance liquid chromatography
HR	heart rate
I	intercept
IC_{50}	C producing 50% of the maximum inhibition
ID	internal diameter
IS	internal standard
iv	intravenous
K	degradation rate constant
K_{10}	rate constant for elimination from central compartment
K_{21}	transfer rate constant from compartment 2 to compartment 1
K_a	apparent absorption rate constant
kcal	kilocalories
K_{e0}	equilibration rate constant
kg	kilogram(s)
L	litre(s)

LA	left atrium
LV	left ventricle
μl	microlitre(s)
μm	micrometer(s)
M	molar
m	meter(s)
MBP	mean blood pressure
MI	myocardial infarction
min	minute(s)
mg	milligram(s)
ml	millilitre(s)
mm	millimetre(s)
mmHg	millimetres of mercury
MRT_o	mean residence time after an oral dose
msec	millisecond(s)
n	number of observations
n	Hill coefficient
ng	nanogram(s)
nm	nanometre(s)
NPM	N-(3-pyrenylmaleimide)
PA	pulmonary artery
PCEA	plasma (angiotensin) converting enzyme activity
po	oral
PRA	plasma renin activity
PVR	pulmonary vascular resistance

r	correlation coefficient
RA	right atrium
rpm	revolutions per minute
s	second(s)
S	slope
SBP	systolic blood pressure
SD	standard deviation
SEM	standard error of the mean
sl	sublingual
SVR	systemic vascular resistance
t	time
$t_{1/2}$	elimination half-life
TBP	tributylphosphine
T_{max}	time at which C_{max} is reached
U	Wood units (mmHg/L/min)
V_c	volume of distribution of the central compartment
V_{dss}	volume of distribution at steady state

1. INTRODUCTION

1.1 History of Captopril

The discovery that certain peptides in the venom of the Brazilian viper *Bothrops jararaca* (*Ferreira et al. 1970, Ondetti et al. 1971*) inhibited ACE led to the synthesis of the nonapeptide teprotide which when administered intravenously lowered blood pressure in patients with essential hypertension. Analysis of the inhibitory action of teprotide led to the synthesis of captopril (*Ondetti et al. 1977, Cushman et al. 1977*). Captopril 1-(D-3-mercapto-2-methyl-1-oxopropyl)-L-proline, Capoten®, E.R. Squibb & Sons, Inc.) was the first orally active ACE inhibitor to be introduced into clinical use and is employed in the treatment of hypertension and congestive heart failure (CHF). It was first released for clinical trials in 1977. Captopril was initially approved in the U.S.A. (and in Canada soon thereafter), in 1980, for use in patients with hypertension. Subsequently, in 1984, approval was also granted for marketing of captopril for the treatment of CHF.

1.2 Congestive Heart Failure

CHF can be defined as the pathophysiologic state in which an abnormality of cardiac function is responsible for failure of the heart to pump blood at a rate commensurate with the requirements of the metabolizing tissues during stress or exercise (*Francis et al. 1984*). CHF is frequently caused by a defect in myocardial contraction or, in the case of infants, it may often be a consequence of a congenital cardiac anomaly, such as an atrial or ventricular septal defect or patent ductus arteriosus. These problems eventually lead to an excessive hemodynamic burden, which is initially compensated for, mainly by a) the Frank-Starling mechanism in which an increase in preload or filling volume acts to increase stroke volume, b) myocardial hypertrophy, with or without dilatation, in which the mass of contractile tissue is augmented, or c) increased myocardial contractility brought about by an increased release of norepinephrine by the sympathetic nervous system (*Francis et al. 1984*). However, once these systems are maximally activated and cardiac output is still insufficient, other vasoconstrictor forces such as the renin-angiotensin system are activated leading to an increase in systemic vascular resistance or afterload. The increased resistance leads to further reduction of cardiac output and a vicious circle ensues.

1.3 Role of the Renin-Angiotensin System in CHF

The main sequence of events in CHF and the role of the renin-angiotensin system are represented in Figure 1.1 (*Romankiewicz et al. 1983*). Myocardial failure or other defects cause a reduction in cardiac output, leading to reduced renal perfusion and stroke volume and increased release of noradrenaline. These and other factors, such as volume depletion resulting from treatment with diuretics or sodium restricted diets, prompt the release of renin from the kidney. Renin is a proteolytic enzyme which cleaves its substrate angiotensinogen to yield the inactive decapeptide angiotensin I (AI). AI is in turn converted, by the action of the angiotensin-converting enzyme (ACE, kininase II), to the potent vasoconstrictor octapeptide, angiotensin II (AII). ACE circulates in soluble form but is also anchored by carbohydrate residues to membrane structures (*Ehlers et al. 1989*). The relative importance of circulatory and tissue based ACE in determining hemodynamic effects is unknown (*MacFadyen et al. 1991*).

AII causes vasoconstriction by a direct action on the smooth muscle of arterioles causing an increase in systemic vascular resistance. AII also promotes release of aldosterone from the adrenal cortex leading to sodium retention and volume expansion. These events increase the preload and afterload and result in a further reduction in cardiac output.

Kininase II, an enzyme identical to ACE, is responsible for the degradation of the endogenous vasodilator, bradykinin, which is formed from kininogen through the action of the enzyme kallikrein. Bradykinin may also increase the release of vasodilatory prostaglandins. Bradykinin may therefore contribute to the reduction of the afterload on the heart.

1.4 Pharmacology of Captopril

The pharmacological properties and therapeutic efficacy of captopril in CHF have been reviewed by Romankiewicz et al. (*1983*) and Brogden et al. (*1988*).

The primary mechanism by which captopril exerts its effects is by inhibiting ACE, the enzyme responsible for the conversion of AI to AII. The inhibitory effects of captopril on the renin-angiotensin-aldosterone system have been demonstrated (*Atkinson et al. 1979, Atlas et al. 1979*). Since AII acts mainly on the arterial bed, captopril causes arterial vasodilation. Captopril also reduces sodium and water retention by the kidneys, because it indirectly reduces aldosterone secretion.

Inhibition of ACE (kininase II) should lead to increased levels of bradykinin. Captopril has been shown to potentiate the effects of exogenous bradykinin in lowering blood pressure (*Donker et al. 1979*).

Levels of vasoactive prostaglandin E_2 and prostacyclin may also be increased during treatment with captopril (*Nishimura et al. 1987, Omata et al. 1987*). Concomitant administration of indomethacin, which inhibits prostaglandin synthesis, markedly attenuates the blood pressure lowering response to captopril in hypertensive patients (*Witzgall et al. 1982a,b*). This suggests that prostaglandins may partially mediate the hemodynamic response to captopril.

1.4.1 Use of Captopril in CHF

Captopril antagonizes the excessive degree of systemic vasoconstriction which is characteristic of CHF, as well as the exaggerated neurohormonal response to the decline in cardiac performance and thus produces circulatory and symptomatic improvement in patients with CHF (*Packer 1987*).

Captopril lowers right atrial, pulmonary arterial and pulmonary capillary pressures. Heart rate is slightly decreased and cardiac output is increased. Systemic vascular resistance is decreased and blood pressure tends to return to baseline with long-term administration (*Gavras et al. 1978, Levine et al. 1980, Ader et al. 1980, Romankiewicz et al. 1983*). Lowering of venous tone and arterial dilatation, as a result of captopril treatment, have both been demonstrated (*Packer et al. 1983, Faxon et al. 1981, Creager et al. 1981, Awan et al. 1981*). In addition, plasma aldosterone, catecholamines and antidiuretic hormone concentrations are decreased (*Kluger et al. 1982*). Thus, the beneficial effects of captopril treatment, in patients with

CHF, have been well documented.

1.5 Use of Captopril in Infants and Children

In one study (*Mirkin et al. 1985*) involving 73 hypertensive children, aged 11 days to 15 years (mean 7.9 years), captopril, usually used in conjunction with other drugs such as diuretics and/or β-blockers, was shown to significantly reduce blood pressure over periods of up to 12 months. Similar results were obtained in other studies (*Sinaiko et al. 1983*, *Bouissou et al. 1986*) on patients with hypertension. In another long term study (*Callis et al. 1986*) of 42 hypertensive children, aged 1 to 17 years (mean 11.2 years), with chronic renal failure, systolic and diastolic pressures were significantly reduced using oral captopril doses ranging from 0.3 to 3 mg/kg/day. Captopril has also been successfully used in neonates with hypertension (*Bifano et al. 1982*, *Hymes et al. 1983*, *O'Dea et al. 1988*), in children with renal hypertension (*Šagát et al. 1986*) and also hypertension due to transplant renal artery stenosis (*Gagnadoux et al. 1985*).

Since 1986, several reports have appeared on the beneficial hemodynamic effects of captopril in the treatment of CHF in infants (*Scammell et al. 1987* and *1989*, *Montigny et al. 1989* and *Shaddy et al. 1988*) and in children (*Girardet et al. 1986*, *Stern et al. 1990*). The beneficial effects demonstrated include such factors as reduction in heart rate and respiratory rate (*Scammell et al. 1987*), reduction in ventricular left to right shunting (*Montigny et al. 1989*, *Shaddy et al. 1988*), significant reduction in diastolic and systolic blood pressures (*Scammell et al. 1989*), decreases in ventricular end-diastolic diameter and left ventricular end-systolic diameter (*Girardet et al. 1986*, *Stern et al. 1990*).

Despite numerous reports of the use of captopril to treat children and infants with hypertension and CHF, only one group (*Sinaiko et al. 1983*) has studied the pharmacokinetics of captopril in children. The kinetics of captopril were studied in 6 children, aged 11-20 years, with renal failure. It may be inappropriate to assume that kinetic information obtained in adults or older children is applicable to infants with CHF. The pharmacokinetics of drugs may be different in infants than they are in adults, since infants are continuously undergoing maturational changes in the physiological functions affecting drug disposition. For example, drug absorption may be impaired due to elevated gastric pH and erratic gastrointestinal motility in infants. Concentrations of most of the hepatic enzymatic microsomal systems responsible for the metabolic degradation of drugs are considerably lower in neonates than in adults and renal function would depend on the developmental stage of the infant (*Morselli 1989*). These physiological factors may contribute not only to differences between adults and infants but also to considerable variability in drug kinetics and dynamics within the infant population. Hence the pharmacokinetics of captopril must be studied, in infants with CHF, if more rational use is to be made of the drug in this patient population.

1.6 Side Effects of Captopril

Captopril is generally well tolerated. Initially, captopril was more frequently used in patients with severe hypertension and concomitant renal function impairment and dosages (more than 150 mg/day) used were considerably higher than those in present use. This resulted in a relatively high incidence of side effects such as rash and dysgeusia. However with the lower doses (less than 150 mg/day) presently used in increasing numbers of patients with mild to moderate hypertension, with normal or near normal renal function, the incidence of side effects is considerably lower (*Edwards et al. 1987, Jenkins et al. 1985*).

The most commonly observed side effects include taste disturbance and skin rash. Taste disturbance was found to occur in 2.2% of patients receiving the lower doses and in 3.4% of patients receiving the higher doses of captopril (*Jenkins et al. 1985*). Skin rashes have been pruritic, erythematous, macular and papular eruptions and occur in up to 6.9% of patients (*Edwards et al. 1987, Jenkins et al. 1985, Groel et al. 1983*). 0.5% of patients exhibit proteinuria (*Jenkins et al. 1985*) and up to 5.4% of patients experience dizziness or vertigo, symptoms of hypotension (*Jenkins et al. 1985*). The latter side effect may be reduced by starting treatment with a low dose of captopril (*Whitworth et al. 1982*). Observations made at the University of Alberta Hospital (Edmonton, Canada) suggest that captopril may cause irritability in a significant number of infants under treatment with captopril. Other side effects such as dry cough and neutropenia are relatively infrequent and generally resolve on discontinuation of captopril treatment.

1.7 Determination of Captopril in Biological Fluids

The first reliable methods for the determination of captopril concentrations in biological fluids involved the use of radiolabelled (^{3}H, ^{14}C or ^{35}S) captopril and thin-layer chromatographic separation procedures (*Kripalani et al. 1980b, Migdalof et al. 1980, Wong et al. 1978*). Clearly, non-radioactive analytical methods were needed, since methods using radiolabelled drug tend to measure total radioactivity and not parent drug and metabolites as separate entities. Also, radioactive analytical methods would not be applicable in the normal clinical situation.

Captopril contains a free sulphydryl group and is unstable in biological fluids. If captopril is incubated in whole human blood at 24°C, approximately 10% is oxidised within 5 minutes (*Kawahara et al. 1981*). Also, captopril does not contain a chromophore. These two problems have generally been dealt with through the use of antioxidants, chelating agents and derivatizing agents. Funke et al. (*1980*) used N-ethylmaleimide to derivatize captopril, in order to minimize oxidation. This was followed by methylation of the derivative and measurement of the methyl ester by gas-liquid chromatography/selected ion monitoring mass spectrometry. This method was modified (*Cohen et al. 1982*) and the speed of analysis was improved. Later, the N-ethylmaleimide derivative was converted to a hexafluoropropanyl ester instead of the methyl ester and was determined using gas chromatography with detection by electron capture (*Bathala et al. 1984*), flame photometry (*Matsuki et al. 1980*), a nitrogen sensitive detector (*Mäntylä et al. 1984*) or selected ion monitoring (*Drummer et al. 1984a, Matsuki et al. 1982*). The lower quantitation limit of most of these techniques is about 10 ng/ml or better.

The introduction of HPLC methods made the measurement of captopril concentrations more generally accessible. The first method was based on derivatization of the sulphydryl group with N-pyrenylmaleimide. The extracted derivative was separated by HPLC and detected using fluorescence detection (*Jarrot et al. 1981*). The detection limit of this method is about 30 ng/ml using 5 ml of blood per sample. In other HPLC assays, derivatization with p-bromophenacyl bromide (*Kawahara et al. 1981*) or N-(4-benzoylphenylmaleimide) (*Hayashi et al. 1985*) for detection by ultraviolet absorption or derivatization with N-(4-dimethylaminophenylmaleimide) followed by electrochemical detection (*Shimada et al. 1982*) was used. Two other HPLC methods (*Perrett et al. 1982, 1984*) differ from each other in the type of electrode used in an electrochemical detector and depend on deproteination, acidification and cooling of the sample (instead of derivatization) to stabilize captopril.

There have also been radioimmunoassay (*Duncan et al. 1983, Tu et al. 1984*) and enzyme immunoassay (*Kinoshita et al. 1986*) techniques developed for the determination of captopril in biological fluids.

Several of these methods (*Kawahara et al. 1981, Jarrot et al. 1981, Perrett et al. 1982, Hayashi et al. 1985, Cohen et al. 1982, Drummer et al. 1984a, Duncan et al. 1983*) have, in effect, been precluded from use in clinical studies involving infants because of the volumes of blood (2-15 ml) required for each measurement. Another limitation on the use of a particular method in a clinical setting is the degree of complexity of the sample preparation. Some of the above methods (*Kawahara et al. 1981, Shimada et al. 1982, Hayashi et al. 1985, Matsuki et al. 1980, Drummer et al. 1984a*) require several (2-8) extraction steps. It would appear that a simple method for the determination of captopril, requiring relatively small volumes of blood, would be desirable.

1.8 Pharmacokinetics of Captopril

The pharmacokinetics of captopril have been reviewed by Duchin et al. (*1988*) and Brogden et al. (*1988*). Information on the disposition and metabolism of captopril has also been reviewed by Drummer et al. (*1986*).

1.8.1 Absorption

Duchin et al. (*1982*), report that after administration of intravenous (iv) and oral doses of radiolabelled captopril to 5 healthy, fasting subjects the absolute bioavailability of captopril was about 65%, based on the ratio of oral versus iv blood AUC values for unchanged captopril (measured by thin-layer radiochromatography). An earlier study (*Kripalani et al. 1980a*) also reports that, based on excretion of radioactivity in urine, approximately 68% of a single oral radiolabelled dose was absorbed after administration to 10 healthy subjects. In humans, 18% of an oral, radiolabelled dose appeared in the faeces in 5 days (*Kripalani et al.*

1980a), probably representing unabsorbed drug since only 0.4% of a 10 mg iv dose of ^{14}C-captopril was excreted in the faeces of 5 healthy subjects in 4 days (*Duchin et al. 1982*).

Absorption of captopril occurs fairly rapidly, with detectable concentrations in plasma within 15 minutes after an oral dose (*Kripalani et al. 1980a*). In healthy subjects, T_{max} occurred at 0.7-0.9 h after administration of 10-100 mg captopril (*Duchin et al. 1982, Kripalani et al. 1980a, Singhvi et al. 1982a, Onoyama et al. 1981*). After single 100 mg doses in these studies, the C_{max} for unchanged captopril ranged between 600 and 900 ng/ml. The pharmacokinetics of unchanged captopril appear to be linear over a wide range of doses (*Duchin et al. 1988*). In patients with CHF, Shaw et al. (*1985*) report that T_{max} varied from 0.75-4 h. The mean AUC, corrected to a dose of 100 mg, was 1408 ng.h/ml and is within the range (corrected to a 100 mg dose) gathered from 3 other studies involving hypertensive subjects with normal renal function (603-2619 ng.h/ml) and is similar to AUC values (also corrected to a 100 mg dose) from 4 other studies involving healthy volunteers (958-1320 ng.h/ml) (*Drummer et al. 1986*).

Ingestion of food can reduce the bioavailability of captopril by 35-55% (*Singhvi et al. 1982b, Mäntylä et al. 1984*). Co-administration of antacid also decreases the bioavailability of captopril by approximately 45% (*Mäntylä* *et al. 1984*).

1.8.2 Distribution

Whole-body autoradiography of ^{14}C-captopril in rats demonstrates widespread distribution of captopril to most tissues except the brain (*Heald et al. 1977*). Captopril rapidly enters the red blood cells and is found in concentrations similar to those in plasma (*Drummer et al. 1983b*).

It is presently unclear whether captopril crosses the placenta in humans (*Boutroy et al. 1984*, *Fiocchi et al. 1984*) although this has been demonstrated in guinea pigs, ewes and rabbits (*Davidson et al. 1981*, *Pipkin et al. 1982*). Captopril does not enter breast milk in humans to a significant extent (*Devlin et al. 1981*).

[^{14}C]Captopril is approximately 30% bound to protein, as determined by equilibrium dialysis using redissolved, methanol precipitated proteins (*Park et al. 1982*). Captopril is principally bound to albumin (*Wong et al. 1981*) in human blood. The binding is covalent but the disulphide bond may be broken enzymatically *in vivo*, releasing captopril (*Maeda et al. 1979*, *McKinstry et al. 1978*). V_{dss} values were 0.70 to 0.75 L/kg in healthy humans (*Duchin et al. 1982*, *Singhvi et al. 1982*). In monkeys, the volume of distribution was estimated to be 3.6 L/kg and in dogs, 2.5 L/kg (*Singhvi et *al. 1981*).

1.8.3 Metabolism

A summary of the known biotransformation processes in humans and animals is shown in Figure 1.2.

Captopril is primarily oxidised to its disulphide dimer and other mixed disulphides. This has been demonstrated *in vitro* in whole blood or plasma of rats, dogs and humans (*Heel et al. 1980*, *Wong et al. 1981*, *Komai et al. 1981*). In man about 50% of a dose of captopril is metabolised, mainly to inactive disulphides with low molecular weight endogenous thiols (glutathione, cysteine) or with proteins (*Drummer et al. 1986*). In humans, the drug is excreted mainly as unchanged captopril (24-46%) (*Kripalani et al. 1980a,b*, *Duchin et al. 1982*, *Singhvi et al. 1982*) and as the cysteine-captopril disulphide (45%), including metabolised fragments of glutathione. The disulphide dimer of captopril (1.5-6%) (*Wong et al. 1978*, *Kripalani et al. 1980a,b*, *McKinstry et al. 1980*) and S-methyl captopril (1% of dose at steady state) (*Drummer et al. 1982*) are minor urinary metabolites. N-acetyl cysteine-captopril disulphide accounts for 25% of urinary metabolites in the dog (*Drummer et al. 1986*). The captopril-glutathione disulphide has not been found in human urine (*Duchin et al. 1988*). The sulphoxide metabolite has been detected in the urine of dogs (5%) (*Komai et al. 1981*) and monkeys (11%) (*Migdalof et al. 1984*), but not in man or rats (*Drummer et al. 1984a*).

The disulphide dimers of captopril may be reduced to some extent by both enzymatic and non-enzymatic processes (*Lan et al. 1982, Yeung et al. 1983*). This reduction was demonstrated *in vivo* (*Park et al. 1982, Breckenridge et al. 1982*) when iv administration of purified plasma protein-bound ^{14}C-captopril to rats resulted in the formation of captopril and low molecular weight disulphides suggesting the dissociation of the protein complex. In addition, both oral and iv administration of captopril disulphide dimer to rats resulted in the appearance of unchanged captopril in blood and inhibition of plasma ACE. Administration of 10 mg/kg captopril disulphide dimer to 4 rats resulted in a C_{max} of 157 ± 30 ng/ml for monomeric (unchanged) captopril as compared with a C_{max} of 678 ± 466 ng/ml after unchanged captopril (10 mg/kg) was administered (*Drummer et al. 1984b, 1985*).

The disulphide metabolites of captopril are found in the highest concentrations in red blood cells but are also widely distributed in all peripheral tissues with the greatest amount found in the kidneys and lung, followed by the spleen and the heart, and least of all in the liver (*Drummer et al. 1983b*). S-methyl captopril is also widely distributed in all peripheral tissues, which is consistent with the wide distribution of the enzymes capable of S-methylating thiols, the thiol methyl transferases (*Drummer et al. 1983a, Weinshilboum et al. 1979*).

1.8.4 Excretion

Urinary excretion of captopril is by a combination of glomerular filtration and active tubular secretion and 94% of captopril excreted appears in the urine within 6 h after administration (*Singhvi et al. 1982*). Co-administration of probenecid, which inhibits tubular secretion of organic acids in the proximal tubule (*Weiner et al. 1964*), reduced renal clearance of captopril by 44% and total body clearance by 19% (*Singhvi et al. 1982*). Using a one-compartment open pharmacokinetic model, Onoyama et al. (*1981*) found a significantly longer $t_{1/2}$ for captopril in patients with chronic renal failure than in normal subjects (0.74 ± 0.09 h versus 0.35 ± 0.02 h). This group also reports that the cumulative urinary excretion of unchanged and total captopril in patients with renal failure was 20.4% and 27.7%, respectively, of that in the normal group over a 6 hour period, with no significant change in AUC, K_a or C_{max} of unchanged captopril. Based on these observations Onoyama et al. (*1981*) suggest that the dosage of captopril should be reduced in patients with renal failure. However, since the apparent oral clearance of captopril did not change and the usual dosing interval (8 h or more) is long relative to the estimated $t_{½}$ of captopril in plasma, the need for a reduction in dosage does not appear to be substantiated.

1.8.5 Summary of Pharmacokinetic Parameters

The oral bioavailability of captopril is about 65 %. T_{max} in healthy subjects varies from 0.7-0.9 h with C_{max} values ranging from 600 to 900 ng/ml after a 100 mg oral dose. Captopril's V_{dss} has been calculated to be 0.70-0.75 L/kg. Estimates of $t_{½}$ for captopril range from 0.35 h (*Onoyama et al. 1981*) to 1.9 h (*Duchin et al. 1982*). In patients with CHF estimates range from 1.06 h (*Cody et al. 1982*) to 7 h (*Shaw et al. 1985*). In the dog and monkey, $t_{½}$ was estimated to be 2.8 h and 2.2 h, respectively (*Singhvi et al. 1981*). Total body clearance in humans, was estimated to be 0.8 L/kg/h and renal clearance 0.4 L/kg/h (*Singhvi et al. 1982*). The average total body clearance and the renal clearance of captopril were 0.605 L/kg/h and 0.341 L/kg/h in the dog and 1.135 L/kg/h and 0.944 L/kg/h in the monkey (*Singhvi et al. 1981*).

1.9 The Relationship between Pharmacokinetics and Pharmacodynamics

The relationship between drug concentration and clinical efficacy and toxicity is of major importance in therapeutics. Pharmacodynamics is the study of the relationship between drug concentration and response. Pharmacokinetics is the study of the time course of absorption, distribution, metabolism and excretion of drugs, that is, the time course of drug concentration. This is usually studied in plasma and/or urine. Integration and application of pharmacokinetics and pharmacodynamics to describe the overall relationship between dose and effect can be very helpful in the proper design of dosage regimens.

Drug action is generally considered to be mediated by effects on specific receptors. If drug concentration at the receptor site is in rapid equilibrium with concentration in plasma and the drug-receptor interaction is reversible, then the relationship between concentration and effect is direct and may be described by a simple pharmacodynamic model. If the drug acts on more than one receptor, it may become necessary to introduce another parameter into the model in order to take this factor into account. In some cases the effect measured either occurs or does not occur (for example, the presence or absence of seizures) and in these cases a threshold concentration of drug must be considered.

However, often plasma drug concentrations are not directly related to the effects observed. For example, development of tolerance or sensitization to a drug would result in different responses to a particular concentration at different times. There may also be an equilibration delay before the drug reaches the site of action. The observed effect (for example, the hypotensive effect of captopril) may be a consequence of a series of biochemical events initiated by the drug. The drug may have more than one site of action and the site or sites may be unknown. In such cases, where the relationship between concentration and effect is indirect, more complex pharmacokinetic-pharmacodynamic models must be used. One approach relates estimated concentrations at a hypothetical effect site to the observed effect (*Sheiner et al. 1979*). This actual site or sites of action are unknown and the hypothetical 'effect site' is simply a compartment introduced into the model to account for a delay between peak concentration and peak effect.

These considerations have led to the development of a variety of models to describe the relationship between drug concentration and effect.

1.9.1 Pharmacodynamic Models

The following are some of the more commonly used pharmacodynamic models:

Fixed effect model: With this model, concentration is related to an effect which either occurs or does not occur. The model has only one parameter, that is, concentration at which the effect appears or the probability that a given effect will occur at a given concentration.

E_{max} model: This empirical model is a hyperbolic function that has been extensively used in enzyme kinetics. With this model, the relationship between effect and concentration is given by:

$$E = \frac{E_{max} * C}{EC_{50} + C}$$

where E = effect, C = drug concentration, E_{max} = maximum effect attributable to the drug and EC_{50} = concentration producing 50% of E_{max}.

If effect, as percentage of E_{max} is plotted against concentration, the curve predicted by the model is hyperbolic. It therefore describes 2 important properties of drug effect. Firstly, as concentration increases, effect tends to a maximum and progressively higher concentrations of drug would be required to increase the effect by a given amount. Secondly, the model predicts no effect when drug concentration is zero.

We find that at low concentrations, effect increases linearly with concentration from about 0-20% effect. In this low concentration range, the simple linear model, described below, could be applied. Effect increases linearly from about 20-80% as concentration increases logarithmically. (When the effects studied are within 20-80% of E_{max}, the log linear model described below, may be used.) Thereafter at high concentrations, effect tends to a maximum as concentration increases.

The model may be modified to incorporate a baseline effect. This would be appropriate in situations where the observed effect is measured as a change from the effect which would normally be present even without the influence of the drug, for example, blood pressure. If the baseline effect is well known, it can be subtracted from the effect.

$$E - E_0 = \frac{E_{max} * C}{EC_{50} + C}$$

where E_0 = baseline effect.

If E_0 is not reliably known it should be measured, and the model becomes:

$$E = \left[\frac{E_{max} * C}{EC_{50} + C} \right] + E_0$$

Inhibitory E_{max} model : The E_{max} model may be modified to describe inhibition of some physiological process by a drug and written as:

$$E = E_0 - \left[\frac{E_{max} * C}{IC_{50} + C} \right]$$

where E_{max} = maximum inhibition of the process that can be attributed to the drug and IC_{50} = concentration producing 50% of the maximum inhibition.

Fractional E_{max} model: In cases where the drug is capable of completely eliminating an effect then, when the effect of the drug is maximum, the baseline effect is totally inhibited and E_{max} in the inhibitory E_{max} model is equal to E_0 and the above equation becomes:

$$E = E_0 * \left[1 - \frac{C}{IC_{50} + C} \right]$$

The expression $C/(IC_{50} + C)$ is the fractional E_{max} model. The term 'fractional' is applied because the model describes the relationship between concentration and the fraction of maximal effect that can be attributed to the drug (*Holford et al. 1981*).

Sigmoid E_{max} model: If a plot of effect versus concentration is S-shaped rather than the hyperbola predicted by the simple E_{max} model, then the 2 parameters of the E_{max} model, i.e. E_{max} and EC_{50} are not enough to describe the relationship between concentration and effect. In this case the Hill equation (*Hill 1910*) may be applied to give the relationship:

where *n* is a number which may be found empirically which

$$E = \frac{E_{max} * C^n}{EC_{50}^n + C^n}$$

affects the slope of the curve.

Linear model: When drug concentrations used are small compared with the EC_{50}, then the increase in effect would be linear with the increase in concentration. This model would therefore be defined as:

$$E = S * C$$

where S = the slope of the line relating concentration and effect. This model predicts no effect in the absence of drug but does not predict a maximum effect. A baseline effect may be incorporated into the model and the equation becomes:

$$E = (S * C) + E_0$$

Log linear model: As mentioned above, between 20% and 80% of maximum response, the increase in effect is linear as concentration increases logarithmically. This gives rise to the relationship:

$$E = (S * \log C) + b$$

where b = value of the intercept on the log concentration axis obtained by extrapolating the linear regression of the effect versus log concentration to zero effect.

This model accounts for the graded response produced by many drugs, that is, as dose is increased within limits, so is effect. However the model does not account for maximum effect and it is not possible, therefore, to state whether the range being examined is between 20% and 80% of the maximum response, that is, the range within which the model holds. The model also does not accommodate a baseline effect (*Holford et al. 1982*).

1.9.2 Pharmacokinetic-Pharmacodynamic Models

One could avoid the necessity of using a pharmacokinetic model by simultaneously measuring effect and concentration at the effect site. However, this is not usually possible. If the effect site is in the central compartment, or if the drug reaches the effect site virtually instantaneously from the central compartment and the effect is immediately triggered, then drug concentration in plasma is directly related to effect since plasma and effect site concentrations are in equilibrium.

Often, however, the time course of drug concentration, as described by a pharmacokinetic model, is not directly related to the observed effect. This may be due to an equilibration delay before the drug distributes into the site of action; or the observed effect may be the result of a series of events initiated by the drug; or the drug may have more than one, known or unknown, site of action. In such cases a combined pharmacokinetic-pharmacodynamic model may be used to generate concentrations at a hypothetical effect site. These estimated concentrations may then be related to the observed effect using a pharmacodynamic model.

A plot of effect versus plasma concentration may be useful in detecting delays in equilibration between drug in the central compartment and effect site. An equilibration delay would produce an anti-clockwise hysteresis; that is, if effect versus time points were joined in chronological order an anti-clockwise loop would be formed because the same concentration at a later time would be associated with a greater effect. This could also be produced if an active metabolite is formed and there is an increase in the metabolite to parent drug ratio over time.

The effect compartment model, proposed by Sheiner et al. (*1979*) is the most commonly applied pharmacokinetic-pharmacodynamic model. Forrester et al. (*1974*) proposed that the time course of the drug effect itself could be used to define the rate of drug movement into the effect site. The half-life of equilibration can be estimated. Failure to incorporate an estimate of equilibration time in non steady-state pharmacodynamic studies may lead to underestimation of drug potency. If the pharmacokinetic model is known it may be used as an input function to a model of the effect site to describe the time course of drug effect. One may then estimate the rate constant of effect site equilibration. Sheiner et al. (*1979*) proposed to include the effect site in an integrated pharmacokinetic-pharmacodynamic model as a separate compartment related to the central compartment by a first order rate constant which allows the time course of drug accumulation at the effect site to be predicted if plasma concentration is changed. Equations have been presented (*Holford et al. 1981*) describing the effect site concentrations for a variety of pharmacokinetic models.

The limitations of Sheiner's effect compartment model include the inability to separately assess the various factors that may contribute to the kinetics of pharmacologic effect (eg., perfusion, diffusion and partition) and the assumption that no tolerance develops to drug effects during the period of study.

Fuseau et al. (*1984*) have presented an approach to pharmacokinetic-pharmacodynamic modelling that is based on the effect model proposed by Sheiner et al. (*1979*). It estimates the rate constant K_{e0} of the linking model as the value that causes the hysteresis curve to collapse to a single curve that represents the empirical concentration effect relationship. A basic assumption is made that tolerance does not develop.

In order to suppress the hysteresis in the concentration versus effect curve one may a) sample the effect site, b) perform steady state experiments where the effect site is presumed to be in equilibrium with the plasma concentration or c) model the effect as a new kinetic compartment linked to one of the pharmacokinetic compartments by a first order process but receiving only a negligible amount of drug so that it does not affect the rate equation.

A nonparametric approach uses the same link model as c) above. All observation pairs of E and effect site concentration (C_e) are used to estimate K_{e0}, the idea being to choose K_{e0} so that the 2 limbs of the C_e versus E curve are superimposed, that is, K_{e0} minimizes the average of the squared differences between the observed and interpolated E.

Assumptions basic to the model are:

a) Equilibrium between drug at the active site and observed E is rapid relative to all other rate processes of the model.

b) E depends only on current C_e (that is, no tolerance or sensitization).

c) The pharmacokinetic model and its parameters are known.

d) The effect compartment is linked to the central compartment by a linear first order process and K_{e0} is the only additional parameter added by the link model.(*Holford et al. 1981,1982*)

1.10 Relationship between the Pharmacokinetics and Pharmacodynamics of Captopril

The pharmacodynamic effects of captopril may be assessed in several ways. These include:

a) Via hormones and enzymes: For example, ACE activity reflects the amount of conversion of AI to AII (*Brunner et al. 1983*). Plasma renin activity is expected to rise with the reduced conversion of AI to AII and therefore renin activity may also serve as an indirect measure of the effects of captopril.

b) Via resting hemodynamics: The effects of ACE inhibition may be assessed, for example, by the drop in blood pressure in hypertensives or improvement in cardiac performance in patients with CHF. Blood pressure is reduced in healthy volunteers also (*Shepherd et al. 1982*).

c) Via agonist titration: Effects of ACE inhibition may be detected based on pressure response to AI (*Ferguson et al. 1977*).

The various methods by which the effects of captopril may be monitored are not without problems. Plasma ACE, which is the most directly accessible (and therefore the most often reported), is not the only source for conversion. Tissue ACE, in the blood vessels and kidneys for example, may play a more important role. Tissue ACE is not easily accessible for activity measurement and therefore plasma renin activity, plasma AI and AII and plasma aldosterone concentrations should be measured simultaneously to completely evaluate the activity of the system (*Brunner et al. 1985*). Plasma ACE activity is measured by addition of a radiolabelled substrate. However, the degree of inhibition is strongly substrate-dependent (*Brunner et al. 1979*). Therefore the results from different laboratories cannot be directly compared.

Plasma renin activity is more easily measured but renin release may be affected by a variety of factors. For example, plasma renin activity was increased to a smaller extent by ACE inhibitors when propranolol was concomitantly administered (*Belz et al. 1988, MacGregor et al. 1985*). The probable reason for this observation is that release of renin from the juxtaglomerular apparatus is stimulated by the sympathetic nervous system, and this effect is blocked by β-adrenergic antagonists (*Hoffman et al. 1991*).

Although hemodynamic measurements are commonly used, they provide only indirect information about the effects of captopril because of the series of biochemical events that must occur before changes are seen in hemodynamic parameters. Also, especially in the case of infants, it may be difficult to control the effects of external stimuli, such as sudden noises, pain, fear, hunger etc, on hemodynamic parameters.

Agonist titration cannot be done on a routine basis.

Given these problems with monitoring the effects of captopril, it is not surprising that precise knowledge of the relationship between the kinetics and dynamics of captopril is still rather limited and no clear relationship has, as yet, been characterized.

Richer et al. (*1984*), in a study of 10 hypertensive subjects, found that the onset of ACE activity inhibition and of diastolic blood pressure decrease closely followed the rise of captopril's plasma levels, while the onset of plasma renin activity increase was delayed. However, while captopril was almost undetectable in plasma after 3 h, its effects persisted for at least 6 h. Cody et al. (*1982*) and Kubo et al. (*1984*) found a correlation between baseline plasma renin activity and degree of improvement in left ventricular function after administration of captopril to patients with CHF. Shaw et al. (*1985*) found no correlation between peak plasma concentrations of captopril and maximal reduction in systemic vascular resistance in patients with CHF. In one study of 10 hypertensive children (*Sinaiko et al. 1983*), although blood pressure was significantly reduced in all the children, no correlation was seen between the reduction in blood pressure and the captopril dose given. In this study, pretreatment plasma renin activity was not a predictor of antihypertensive response to captopril. Also, there was no correlation between the blood concentrations of captopril and the antihypertensive response.

The relationship between the pharmacokinetics and pharmacodynamics of captopril has not been adequately characterized. This is partially due to the fact that the concentrations of captopril measured in plasma do not directly relate to the effects seen because of the mechanisms by which captopril acts. It is also difficult to decide on a meaningful and measurable end-point with which to relate the kinetics of captopril. Considering also, that there is, as yet, no kinetic data available for captopril in infants, it is clear that more work needs to be done.

1.11 The Piglet as an Animal Model

The use of an appropriate animal model would allow continuous measurement of hemodynamic parameters, in more detail than is usually possible in humans. Continuous measurement permits better characterization of hemodynamic changes over time and is not feasible in human subjects. In addition, the frequency of blood sampling, for plasma drug concentration measurements, would be less restricted in an animal model than in humans. Pigs have long been used in cardiovascular research (*Detweiler et al. 1966*) because of their anatomic and physiologic similarities to humans (*Bustad 1966*). It has been reported that newborn piglets up to 2.5 weeks old correspond to the first 6 months of the human infant (*Boudreaux et al. 1984*). Experimentally induced cardiac failure in swine has also been described (*Maaske et al. 1966, Lumb et al. 1966*). The effect of intravenous captopril on ventricular tachycardia has been studied in Yorkshire swine (*Kingma et al. 1986*). Kingma et al. (*1986*) concluded that captopril reduces the inducibility of sustained ventricular tachycardia one week after experimental myocardial infarction in the pig. Thus it appears reasonable to investigate the piglet as a suitable animal model in which to study the pharmacokinetics and pharmacodynamics of captopril.

1.12 Hypotheses

1. The pharmacokinetic parameters for captopril in infants with CHF are not within the range reported for adults with CHF.
2. The acute hemodynamic effects of captopril are beneficial in infants with CHF.
3. The pharmacokinetics and hemodynamic effects of captopril in the piglet are sufficiently similar to those in the human for the piglet to make a useful animal model.
4. There is a relationship between the pharmacokinetics and the hemodynamic effects of captopril.

1.13 Objectives

1. To develop a simple, sensitive and specific method to determine plasma concentrations of unchanged and dimerized captopril, using small volumes of blood.
2. To determine standard pharmacokinetic parameters for captopril in infants with CHF.
3. To confirm that the acute hemodynamic effects of captopril are beneficial in infants with CHF.
4. To evaluate the piglet as an animal model in which to study the relationship between the pharmacokinetics and hemodynamic effects of captopril.
5. To model the relationship between the pharmacokinetics and pharmacodynamics of captopril.

1.14 Figures

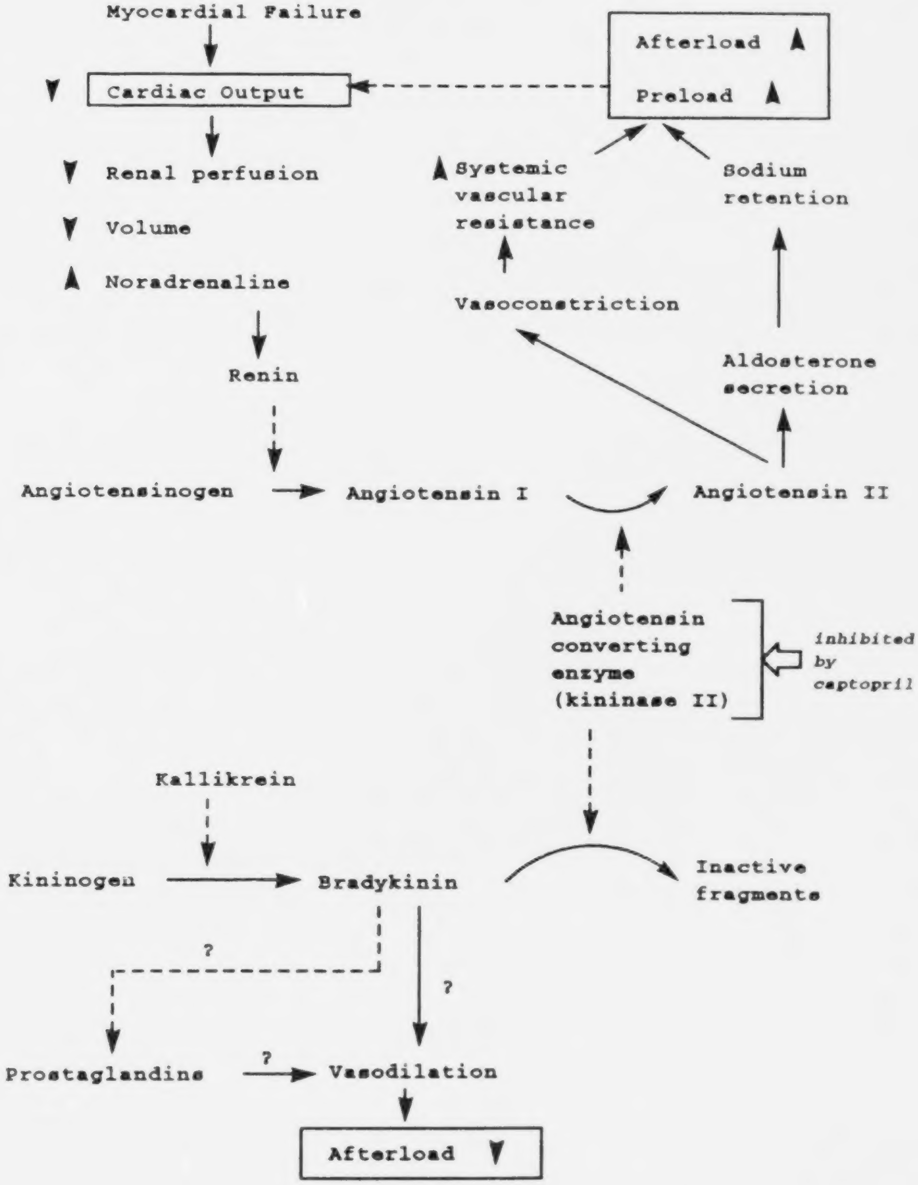
Myocardial Failure
Cardiac Output
Afterload
Preload
Renal perfusion
Volume
Noradrenaline
Systemic vascular resistance
Sodium retention
Vasoconstriction
Renin
Aldosterone secretion
Angiotensinogen
Angiotensin I
Angiotensin II
Angiotensin converting enzyme (kininase II)
inhibited by captopril
Kallikrein
Kininogen
Bradykinin
Inactive fragments
?
?
Prostaglandins
?
Vasodilation
Afterload

Figure 1.1 Simplified representation of the role of the renin-angiotensin-aldosterone system and the kallikrein-kinin-prostaglandin systems in the maintenance of the congestive heart failure state.
(*Romankiewicz et al 1983*)

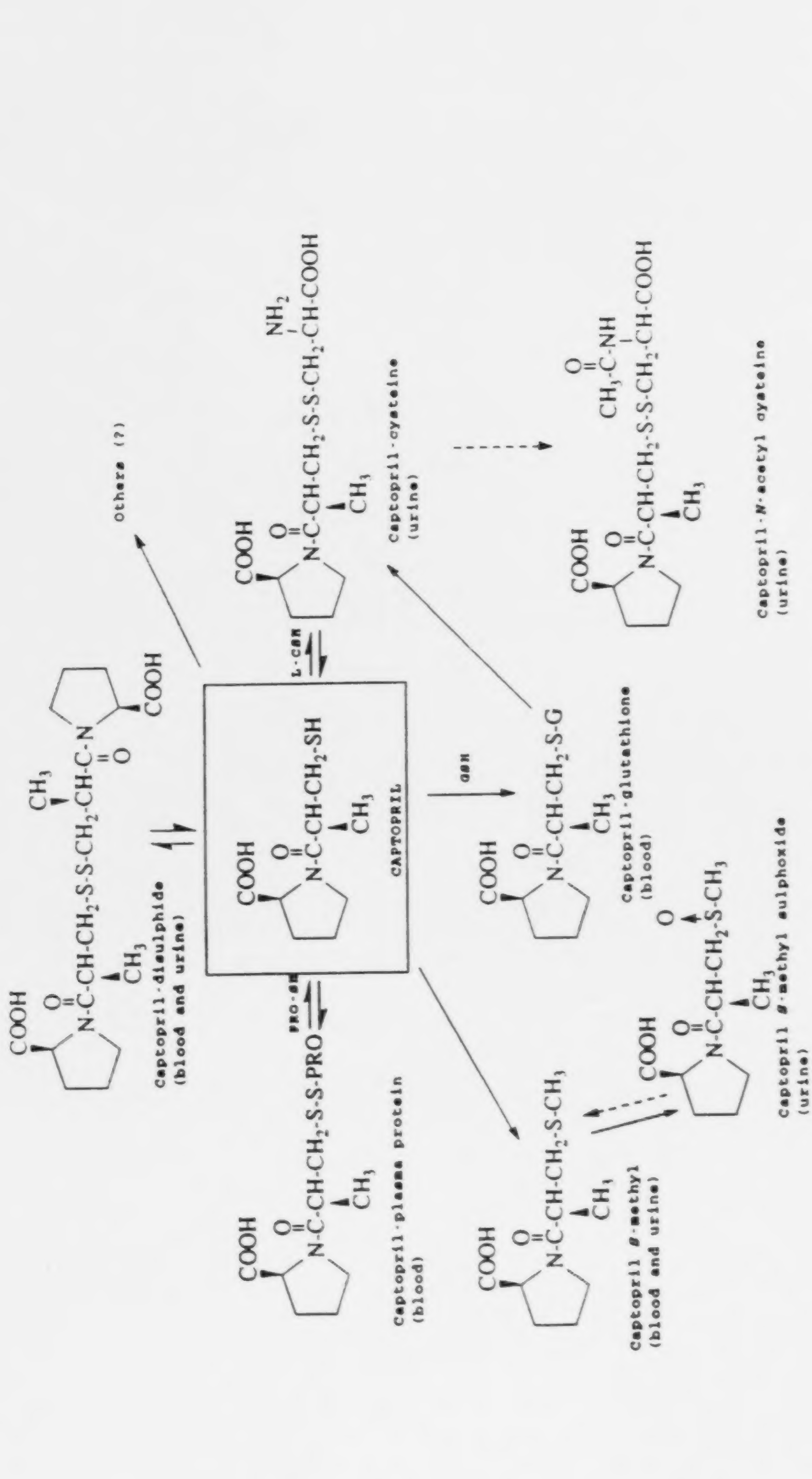
Captopril-disulphide (blood and urine)
CAPTOPRIL
Others (?)
PRO-SH
GSH
L-CSH
Captopril-plasma protein (blood)
Captopril-glutathione (blood)
Captopril-cysteine (urine)
Captopril S-methyl (blood and urine)
Captopril S-methyl sulphoxide (urine)
Captopril-N-acetyl cysteine (urine)

Figure 1.2 Summary of biotransformation of captopril in blood and urine.
(dotted arrows = proposed pathways) (*Migdalof et al 1980*)

1.15 References

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2. Simplified Determination of Captopril in Plasma by High-Performance Liquid Chromatography[1]

2.1 Introduction

Captopril, 1-(D-3-mercapto-2-methyl-1-oxopropyl)-L-proline, is an angiotensin-converting enzyme inhibitor used in the treatment of hypertension and congestive heart failure in adults (*Giudicelli et al. 1984, Cody et al. 1982*) and in children (*Sinaiko et al. 1983, Girardet et al. 1986*). However, the use of captopril in infants has been on an empirical basis, due to the absence of relevant kinetic data. Reasons for this absence may include the complexity of the available assay methods for captopril and/or the large volumes of blood required. Captopril has been determined by several methods, including high-performance liquid chromatography (HPLC) (*Kawahara et al. 1981, Jarrott et al. 1981, Shimada et al. 1982, Perret et al. 1982, Hayashi et al. 1985*), gas chromatography (*Matsuki et al. 1980*), gas chromatography-mass spectrometry (GC-MS) (*Cohen et al. 1982, Drummer et al. 1984*), radioimmunoassay (*Duncan et al. 1983, Tu et al. 1984*) and enzyme immunoassay (*Kinoshita et al. 1986*). The complexity of these methods may limit their applicability and specialized equipment such as GC-MS may not be widely accessible. Also the volumes of blood required may preclude the use of these methods in studies involving neonates. A simple HPLC assay has been developed and was used in a pharmacokinetic study with a 15-month old infant with congestive heart failure. This assay was also adapted to measure captopril in plasma collected in a study involving adult human volunteers.

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2.2 Experimental

2.2.1 Materials

Captopril (SQ 14,225) and captopril disulphide (SQ 14,551) were donated by E.R. Squibb (Princeton, NJ, U.S.A.), (4R)-2-(2-hydroxyphenyl)-3-(3-mercaptopropionyl)-4-thiazolidinecarboxylic acid, internal standard (IS) (SA 446), was donated by Santen Pharmaceutical (Osaka, Japan) (The chemical structures of captopril, captopril disulphide, N-(3-pyrenylmaleimide) (NPM) and IS are shown in Figure 2.1). NPM was purchased from Fluka (Happauge, NY, U.S.A.) and was purified on a column (43 cm × 2 cm) packed with silica gel (E. Merck, Darmstadt, F.R.G.) using chloroform as the eluent. NPM was used as a 1.5 mg/ml solution in acetonitrile. All other chemicals used were of analytical-reagent grade.

2.2.2 Instruments

The high performance liquid chromatograph used was equipped with a Partisil®5 ODS-3 C_{18} column (5 μm, 4.6 mm ID × 100 mm, Whatman, Clifton, NJ, USA). Samples were introduced by means of a Model U6K sample injector (Waters, Mississauga, Canada) or an automatic sample processor (WISP Model 710B, Waters, Mississauga, Canada) and detected by an FS 970 fluorometer (Schoeffel, Oakville, Canada) or a Waters 470 scanning fluorescence detector (Waters, Massachusetts, USA) with the excitation and emission wavelengths set at 340 and 389 nm, respectively. The mobile phase was acetonitrile-0.1 M citric acid buffer at pH 3.1 (38:62) for total captopril analyses and acetonitrile-1% acetic acid (37:63) for unchanged captopril analyses. Each was run at a flow rate of 1.5 ml/min.

Chromatographic separations, for unchanged and total captopril, similar to those achieved with the above system were also attained using:

i) a μ-Bondapak® C_{18} column (10 μm, 3.9 mm ID × 300 mm, Waters, Mississauga, Canada) with a mobile phase consisting of acetonitrile-1% acetic acid (43:57) for unchanged captopril or (42:58) for total captopril. Each was run at a flow rate of 2 ml/min.

ii) a Zorbax® C_8 column (5 μm, 4.6 mm ID × 250 mm, Dupont, Delaware, U.S.A.) with a mobile phase consisting of acetonitrile-potassium phosphate buffer (pH 7) containing 0.01 M or 0.005 M tetramethylammonium chloride (42:58 or

41:59) for unchanged or total captopril determination respectively. Each mobile phase was run at a flow rate of 1 ml/min.

iii) a Separon® C_{18} SGX column (7 μm, 3.3 mm ID × 150 mm, Tessek, California, U.S.A.) with a mobile phase consisting of acetonitrile-potassium phosphate buffer (pH 7) containing 0.01 M tetramethylammonium chloride (32:68) at a flow rate of 1 ml/min for unchanged captopril determination.

2.2.3 Procedures for the determination of captopril in neonatal plasma

2.2.3.1 Unchanged captopril

Freshly drawn blood (1 ml) was mixed with 50 μl of a solution of EDTA (0.1 M) and ascorbic acid (0.1 M) (*Jarrott et al. 1981*). The mixture was immediately centrifuged at 13000 g for 2 min in a microcentrifuge (Model 235A, Fisher, Canada). A 0.5-ml aliquot of the supernate was separated, and 2 ml of a 0.1 M phosphate buffer (pH 7), 200 ng of IS and 0.2 ml of a solution of the derivatizing agent NPM were added. This mixture was shaken at room temperature for 15 min on a vortex shaker (Vibrax VXR2, Janke and Kunkel, Germany) at 1400 rpm and was then acidified with 0.1 ml hydrochloric acid (11 M) and extracted with 6 ml ethyl acetate by vortex mixing for 10 min. After centrifuging at 2500 g for 5 min in a Dynac centrifuge (Clay Adams, USA) the organic layer was removed and dried under nitrogen. The residue was dissolved in 50 or 200 μl of acetonitrile and aliquots of 5-15 μl were injected into the HPLC system.

2.2.3.2 Total captopril (captopril and its mixed disulphides)

A 0.5-ml plasma sample was mixed with 50 μl of a solution of EDTA (0.1 M) and ascorbic acid (0.1 M), 2 ml of 0.1 M phosphate buffer (pH 7), 400 ng IS and 0.1 ml of a 2% solution of tributylphosphine (TBP) in acetonitrile. The mixture was incubated at 50°C for 1 h. After incubation, the mixture was cooled to room temperature by standing the tubes in water and then 0.2 ml of a solution of NPM was added. This mixture was shaken at room temperature for 15 min and then extracted and assayed as for unchanged captopril.

2.2.4 Calibration curves for unchanged and total captopril

Calibration curves for unchanged and total captopril were constructed by spiking 0.5 ml plasma samples with known amounts of captopril and captopril disulphide, respectively, and assaying as described above. The peak-height ratio of captopril to internal standard was plotted against the concentration of captopril.

2.2.5 Patient study

Captopril (1 mg/kg total body weight) was administered as an oral solution to a 15-month-old infant during cardiac catheterization, as part of treatment for congestive heart failure. Blood samples (2 ml) were drawn 0, 0.25, 0.5, 1.0, 1.5, 2, 4, 6 and 8 h after the initial dose. Unchanged and total captopril levels were measured. Informed parental consent was obtained before the study.

2.2.6 Validation study for an adult trial

A separate validation study was conducted, after the present assay was published, in order to establish the accuracy and precision of the assay methods for unchanged and total captopril so that these methods could be used in a trial involving moricizine and captopril. This study of the intra- and inter-day variability of the assay methods did not include the calibration curves mentioned above and the results are reported separately.

2.2.6.1 Unchanged captopril

In order to conduct the intra- and inter-day studies using the same standard solutions it was necessary to prepare batches of derivatized standard solutions in plasma, since underivatized captopril is relatively unstable in plasma (*Kawahara et al. 1981*). IS solutions were freshly prepared for each analysis since IS contains a free sulphydryl group and may therefore be unstable in solution. A fixed concentration was used each time. The stability of the maleimide derivative of captopril in a mixture of plasma and buffer (i.e. in the derivatized standard solutions) was assured by comparing the slopes of standard curves prepared over a period of 6 months with samples prepared using the same procedure as for the samples in the validation study.

Standard samples were prepared by spiking 2-ml aliquots of plasma with appropriate amounts of captopril to yield concentrations ranging from 10 to 1500 ng/ml. To each 2 ml of plasma, 0.2 ml of a solution of EDTA (0.1 M) and ascorbic acid (0.1 M), 8 ml of 0.1 M phosphate buffer (pH 7) and 0.8 ml of a solution of NPM (1.5 mg/ml in acetonitrile) were rapidly added. This mixture was then shaken at room temperature for 15 min. Batches of the various concentrations were then pooled, mixed and frozen. Aliquots (2.75 ml) were used for each standard sample. This volume was used because the volume of plasma, buffer, NPM solution and EDTA and ascorbic acid solution in individual samples added up to approximately 2.75 ml. These standard solutions were prepared once prior to the start of the validation study.

The same procedures as were used in the preparation of the standard samples, were used by a second person to separately prepare quality control samples with concentrations ranging from 10-1500 ng/ml as shown in Table 2.1.

At the time of analysis, samples were equilibrated to room temperature, IS was added and the samples were shaken for 15 min. The standard and quality control samples were then extracted and processed as described above for unchanged captopril.

Concentrations of the quality control samples were estimated by inserting peak height ratios (captopril/IS) into the linear equations of the standard curves. Intra-day precision was calculated based on replicate analyses (n=6) at 6 concentrations. Each concentration was analyzed in duplicate on two occasions and in 6 replicates on the third.

2.2.6.2 Total captopril

For the purpose of the validation study, batches of plasma were spiked with captopril disulphide dimer to yield standard solutions with concentrations ranging between 500-3000 ng/ml. Quality control samples were separately prepared with concentrations ranging from 500-3000 ng/ml as shown in Table 2.2. The stability of samples prepared using this procedure was assured in the same way as for unchanged captopril over a 2 month period.

At the time of analysis, 0.2 ml each of standard and quality control plasma samples was mixed with 0.02 ml of a solution of EDTA (0.1 M) and ascorbic acid (0.1 M), 0.8 ml of potassium phosphate buffer (pH 7), 40 μl of TBP (2% in acetonitrile), and IS. This mixture was treated and analyzed as described above for total captopril.

The intra- and inter-day study and analysis of results was carried out as for unchanged captopril using 4 concentrations for quality control.

2.3 Results and Discussion

Although chromatographic separation of the captopril derivative from endogenous substances proved to be a difficult task, the HPLC assay which we have developed for captopril and its mixed disulphides in plasma is a considerable improvement over available methods. This method makes it possible to obtain pharmacokinetic data for paediatric patients, since only 1 ml of blood or 0.5 ml of plasma is required to determine unchanged or total captopril concentrations. Other methods (*Kawahara et al. 1981, Jarrott et al. 1981, Perret et al. 1982, Hayashi et al. 1985, Cohen et al. 1982, Drummer et al. 1984, Duncan et al. 1983*) require 2-15 ml of blood and this precludes their use with infants due to the small volumes of blood available. Sample preparation is considerably faster and easier than with previous methods (*Kawahara et al. 1981, Jarrott et al. 1981,* *Shimada et al. 1982, Hayashi et al. 1985, Matsuki et al. 1980, Cohen et al. 1982, Drummer et al. 1984, Duncan et al. 1983, Tu et al. 1984, Kinoshita et al. 1986*). Sample treatment in our method involves only one extraction and no other clean-up steps, as compared with 2-8 extractions required by other methods (*Kawahara et al. 1981, Shimada et al. 1982, Hayashi et al. 1985, Matsuki et al. 1980, Drummer et al. 1984*). Nevertheless, typical chromatograms (Figures 2.2 and 2.3) show the maleimide derivatives of captopril and the IS to be well separated from peaks due to endogenous substances. These chromatograms were obtained using a Partisil®5 ODS-3 C_{18} column. Unfortunately, we were not able to reproduce the original separation of captopril and IS from endogenous substances using subsequently purchased 'identical' Partisil®5 ODS-3 C_{18} columns. In addition, the peaks obtained for IS and captopril were broad (peak base > 1.5 min) and split. Although a broad (peak base > 3 min), split peak, for a maleimide derivative of captopril, using a PAK A C_{18} column (8 mm ID × 100 mm, Waters, Chippendale, Australia) has been previously reported (*Jarrott et al. 1981*), the reasons for this observation are still unclear since captopril is supplied as a pure isomer. The reaction of a sulphydryl group with the maleimide moiety would introduce a chiral centre into the maleimide ring, suggesting the possibility that the derivatized product may be in the form of two isomers. Partial separation of such isomers may serve to explain the broad, split chromatographic peak observed for captopril. It is not desirable to separate such isomers since they are formed at the derivatization step and represent a single entity, captopril.

We encountered similar chromatographic difficulties using several other columns, including Nova-Pak C_{18} Radial Pak (4 μm, 5 mm ID × 100 mm, Waters, Mississauga, Canada) and μ-Bondapak C_{18} (5 μm, 8 mm ID × 100 mm, Waters, Mississauga, Canada) columns. Eventually, single, well-separated peaks for captopril and IS were obtained using a μ-Bondapak C_{18} (10 μm, 3.9 mm ID × 300 mm, Waters, Mississauga, Canada) column.

At this point, this assay was applied to a project undertaken by our laboratory which involved the determination of unchanged and total captopril in plasma samples from healthy, adult humans to study possible pharmacokinetic interaction between captopril and the antiarrhythmic drug moricizine. Plasma samples from subjects receiving captopril and moricizine together, showed a peak which interfered chromatographically with captopril. Plasma samples from subjects receiving moricizine alone did not show this peak. This interfering peak, which was not seen when plasma was spiked with captopril and moricizine (Figure 2.4), could not be separated using the μ-Bondapak® C_{18} (10 μm) column (See Figure 2.5). This led to a search for other columns suitable for this assay. Use of tetramethylammonium chloride, an ion-pairing reagent, with the Zorbax® or Separon® columns (described in section 2.2.2) produced appropriate separations of captopril and IS from this interfering peak and also gave single peaks (with peak base < 1.5 min), for both captopril and IS, that permitted accurate quantitation of low drug concentrations (10 ng/ml and 50 ng/ml for unchanged and total captopril respectively). Representative chromatograms, obtained using a Zorbax® C_8 column, for blank plasma and plasma spiked with captopril are shown in Figure 2.6. Representative chromatograms (Figure 2.7), also obtained using a Zorbax® C_8 column, for plasma samples taken from a patient after administration of captopril alone and after simultaneous administration of captopril and moricizine, show the interfering peak to be well separated from the captopril peak.

Other drugs, including chlorpromazine, promethazine, chloral hydrate (pre-catheterization sedative mixture), furosemide and digoxin, used concomitantly with captopril in a study involving infants with congestive heart failure, did not interfere with this assay. Since these drugs are not fluorometrically detectable at the wavelengths used in this assay they will not interfere, regardless of which column is used. Plasma volumes ranging from 0.1 to 1.0 ml can be analyzed without modification to the method.

Derivatization with N-pyrenylmaleimide of thiol compounds in general and of captopril in particular has been described previously (*Weltman et al. 1973, Jarrot et al. 1981*). The derivatives of captopril and IS were found to be stable for at least one month when stored at -20°C. The reduction of the captopril dimer and mixed disulphides with TBP has been shown to be effective (*Kawahara et al. 1981, Hayashi et al. 1985*). EDTA has been found to increase the stability of captopril in a phosphate buffer (pH 6.6) containing cupric acetate, by chelating the trace metal which catalyses the oxidation of captopril (*Lee et al. 1987*). Ascorbic acid has been shown to increase the stability of captopril in dog food by reducing the oxidation of captopril (*Seta et al. 1988*). It is likely that EDTA and ascorbic acid, when added to blood stabilize captopril by these same mechanisms.

In the paediatric study, calibration curves (Figure 2.8) were linear in the range 10-400 ng/ml for unchanged and 50-750 ng/ml for total captopril. The coefficients of variation (n=4 for all points) were 6.6 and 2.8% with 13.6 and 408 ng/ml captopril, respectively, for unchanged captopril and 6.7 and 2% with 55.5 and 740 ng/ml captopril, respectively, for total captopril. The limit of determination was 10 ng/ml for unchanged captopril and 50 ng/ml for total captopril using 0.5 ml plasma.

The maximum sensitivity of reported methods (*Kawahara et al. 1981, Jarrot et al. 1981, Shimada et al. 1982, Perret et al. 1982, Hayashi et al. 1985, Matsuki et al. 1980, Cohen et al. 1982, Drummer et al. 1984, Duncan et al. 1983, Tu et al. 1984, Kinoshita et al. 1986*) ranges from 0.5 to 1000 ng/ml. The coefficients of variation of these assays ranged from 0.6 to 13%. Hence the sensitivity and reproducibility of our method are comparable with available methods. Note that, although other assays have similar quantitation limits, the use of a smaller sample volume provides a definite advantage. Some methods (*Kawahara et al. 1981, Drummer et al. 1984, Duncan et al. 1983, Kinoshita et al. 1986*) have detection limits (0.5-5 ng/ml) lower than ours. However, these methods are more cumbersome and time-consuming. The methods of Kawahara et al. (*1981*) and Drummer et al. (*1984*) also require larger volumes of blood (2-3 ml). The method of Perret and Drury (*1982*) involves fewer steps than ours and has similar detection limits, but it requires 1 ml of plasma and total captopril is not measured. In addition, while underivatized captopril has been shown to be stable in Britton-Robinson buffer solution at pH 2.1 for 4 days (*Kawahara et al. 1981*), these results are not necessarily applicable to deproteinated plasma as captopril is known to degrade faster in plasma (*Kawahara et al. 1981*). Therefore, stability of captopril may be a problem, especially if batches of samples are to be injected using an autosampler at room temperature. Also, as pointed out by the authors, the mercury electrode of the electrochemical detector requires extensive maintenance and the mercury amalgam would be destroyed by ion-pairing agents.

The present assay was used in a study with a 15-month-old infant with congestive heart failure. The plasma concentration versus time curves for unchanged and total captopril are shown in Figure 2.9. After a 1 mg/kg oral dose, the time to reach maximum plasma concentration for both unchanged and total captopril was 1 h. The maximum plasma concentration was 223 ng/ml for unchanged captopril and 412 ng/ml for total captopril.

However, for results obtained using this assay to be acceptable to the pharmaceutical industry, the assay was required to be validated according to good laboratory practice. A validation study was therefore undertaken. The concentration range studied (10-1500 ng/ml) suggest that the results of the validation study for unchanged captopril are directly applicable to the present thesis work. It was found that total captopril concentrations found in the adult human plasma samples in the abovementioned project ranged from 500 to 3000 ng/ml. This range was therefore chosen for the validation study for total captopril. (This study was carried out after sufficient samples had been analyzed to establish the concentration range for total captopril to be examined). The calibration curve for total captopril had previously been shown to be linear, at least from 50 to 750 ng/ml, and the y-axis intercepts of calibration curves from 500 to 3000 ng/ml (see Table 2.3) did not differ systematically from zero. It is therefore suggested that the results of the validation study, carried out for total captopril in the concentration range of 500-3000 ng/ml, would be applicable to lower concentrations.

Samples prepared for the validation study were found to be stable (over a 6 month period for unchanged captopril and a 2 month period for total captopril) since no significant systematic change, over time, was seen in the slopes of the standard curves for total or unchanged captopril. (See Tables 2.3 and 2.4).

The results of the separate validation study were as follows: For unchanged captopril, CV (%) in the intra-day study were 1.8-10.4% with concentrations of 10-1500 ng/ml. (Details are provided in Table 2.1). For total captopril, CV (%) in the intra-day study were 6.4-12.2% with concentrations of 500-3000 ng/ml. (Details are provided in Table 2.2).

In conclusion, we have developed a relatively simple HPLC assay for unchanged and total captopril in plasma. We have validated this assay according to good laboratory practice. This assay is suitable for use in studies involving either infants or adults. We have used this method in a study involving an infant with CHF and in a study of potential interaction between moricizine and captopril in healthy adult subjects.

2.4 Tables

Table 2.1 Inter- and intra-day assay variability for unchanged captopril in quality control samples.

	Captopril Concentration (ng/ml)					
Added	**10.0**	**10.5**	**300.0**	**600.0**	**1000**	**1500**
Found						
Trial 1	10.0	10.5	318.2	580.1	930.9	1522.5
	9.9	10.7	319.8	566.2	1022.8	1680.5
Mean	10.0	10.6	319.0	573.1	976.9	1601.5
Trial 2	10.7	10.5	325.8	632.7	827.5	1323.8
	9.7	10.0	315.1	611.8	831.5	1414.8
Mean	10.2	10.2	320.5	622.3	829.5	1369.3
Trial 3	11.2	11.8	311.1	646.9	926.5	1423.0
(Intra-day)	9.6	11.0	301.9	650.6	980.4	1509.0
	10.8	10.4	316.9	651.3	1071.0	1710.0
	10.0	10.3	312.5	641.9	828.9	1463.0
	11.0	10.6	321.5	623.5	939.9	1451.0
	10.9	10.4	311.2	623.2	1107.0	D
Mean	10.6	10.8	312.5	639.6	975.6	1511.2
SD	0.6	0.5	6.0	11.8	101.6	115.4
% CV	5.7	4.6	1.9	1.8	10.4	7.6

D, sample discarded

Table 2.2 Inter- and intra-day assay variability for total captopril in quality control samples.

	Total Captopril Concentration (ng/ml)			
Added	**500.0**	**550.0**	**1000.0**	**3000.0**
Found				
Trial 1	567.8	758.0	1318.0	3412.0
	485.1	522.5	859.9	2616.0
Mean	526.5	640.3	1089.0	3014.0
Trial 2	613.6	604.0	1130.0	3332.0
	552.2	514.3	1184.0	2995.0
Mean	582.9	559.2	1157.0	3163.5
Trial 3	609.7	615.8	1208.0	3400.0
(Intra-day)	618.4	505.4	1164.0	3274.0
	477.6	558.9	930.0	D
	602.4	544.2	999.5	3366.0
	469.2	546.4	935.6	2978.0
	545.6	560.2	1132.0	3560.0
Mean	553.8	555.1	1061.5	3315.6
SD	67.4	35.8	121.6	215.1
% CV	12.2	6.4	11.5	6.5

D, sample discarded

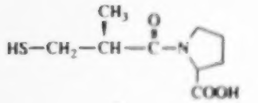
Table 2.3 Compilation of calibration curves for total captopril. I=intercept, S=slope of the regression equation.

Concentration = I + S * peak height ratio			
Time	I	S	r^2
04-Apr-90	338.7	4369.3	0.990
08-May-90	-21.5	3206.6	0.985
09-May-90	84.6	2233.4	0.995
10-May-90	-171.5	3347.3	0.970
12-May-90	109.8	1974.3	0.978
15-May-90	-288.2	3638.4	0.995
16-May-90	-127.3	3050.6	0.995
16-May-90	-68.5	3866.5	0.937
18-May-90	48.3	2679.5	0.959
18-May-90	70.7	3601.3	0.969
21-May-90	118.9	2452.6	0.958
23-May-90	-68.4	3114.6	0.940
24-May-90	88.5	3461.4	0.954
29-May-90	57.0	3716.9	0.964
Mean	12.2	3193.8	0.971
SD	152.6	668.9	0.020
% CV	1250.8	20.9	2.060

Table 2.4 Compilation of calibration curves for unchanged captopril. I=intercept, S=slope of the regression equation.

Concentration = I + S * peak height ratio			
Time	I	S	r^2
03-Oct-89	2.1	673.4	0.996
21-Nov-89	-2.8	792.1	0.998
09-Feb-90	7.3	609.7	0.998
11-Feb-90	9.4	802.4	0.991
15-Feb-90	7.8	584.3	0.992
16-Feb-90	-0.9	602.1	0.994
20-Feb-90	9.1	1317.5	0.999
03-Mar-90	8.1	487.0	0.994
06-Mar-90	2.1	1178.1	0.999
11-Mar-90	0.2	773.4	0.998
12-Mar-90	5.9	712.5	0.995
19-Mar-90	0.8	471.1	0.996
Mean	4.1	750.3	0.996
SD	4.3	258.3	0.003
% CV	104.9	34.4	0.3

2.5 Figures



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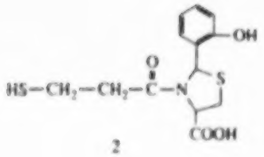

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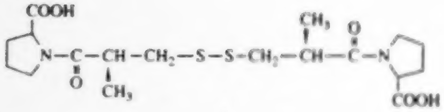

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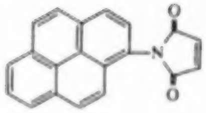

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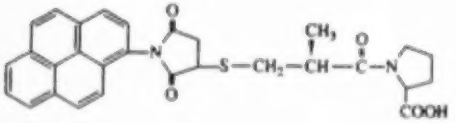

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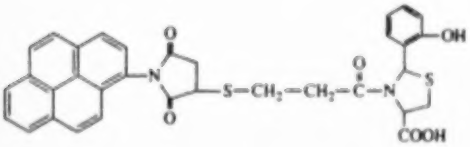

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Figure 2.1 Chemical structures of captopril (1), internal standard (2), captopril disulphide (3), N-(3-pyrenylmaleimide) (4) and proposed chemical structures of captopril-maleimide (5) and internal standard-maleimide (6) derivatives.

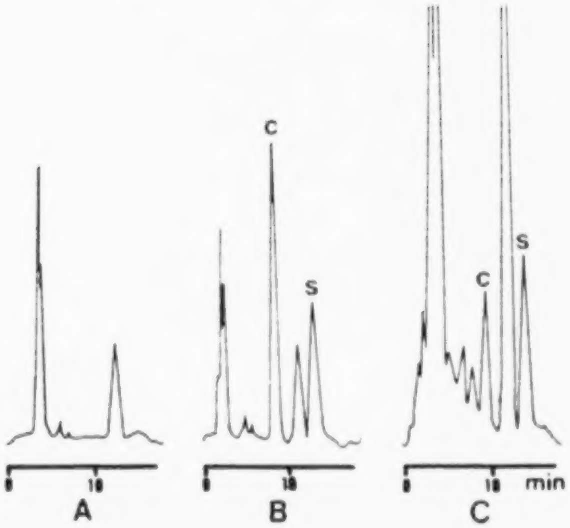

Figure 2.2 Typical chromatograms for unchanged captopril in plasma samples, using a Partisil® ODS-3 C_{18} column. (A) blank; (B) spiked with 476 ng/ml captopril; (C) patient. Peaks: c = captopril, s = internal standard.

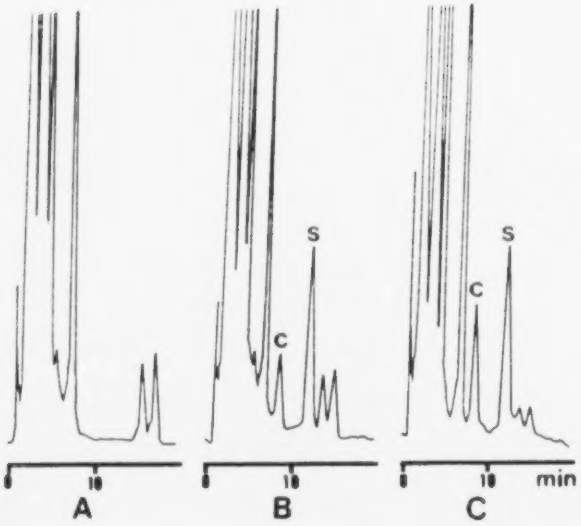

Figure 2.3 Typical chromatograms for total captopril in plasma samples, using a Partisil® ODS-3 C_{18} column. (A) blank; (B) spiked with 162 ng/ml unchanged captopril equivalent; (C) patient. Peaks: c = captopril, s = internal standard.

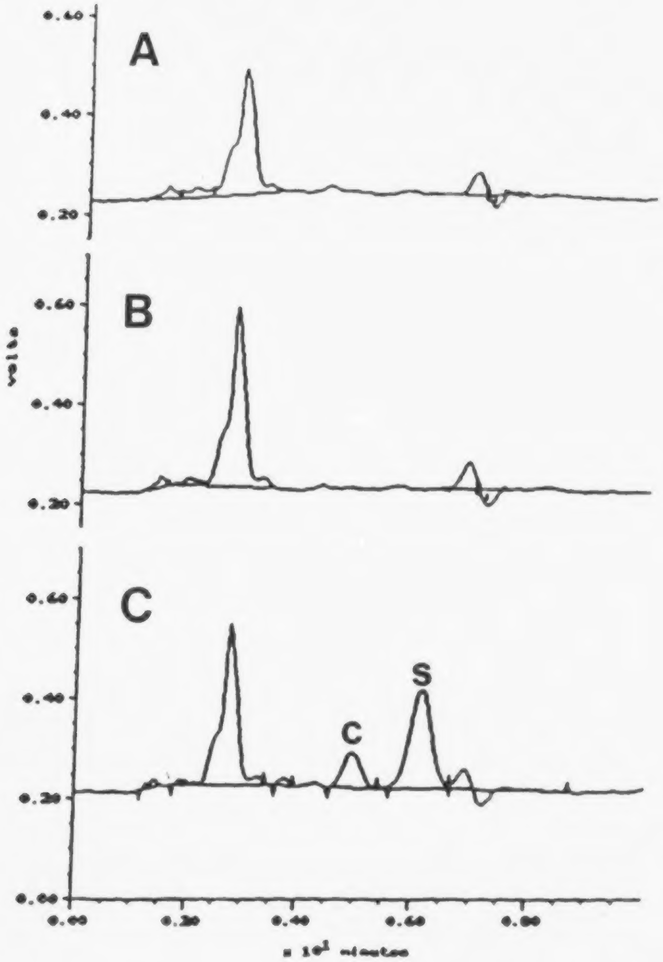

Figure 2.4 Representative chromatograms, obtained using a μ-Bondapak® C_{18} (10 μm) column, for plasma samples. (A) blank; (B) spiked with 760 ng/ml moricizine; (C) spiked with captopril, internal standard and 760 ng/ml moricizine. Peaks: c = captopril, s = internal standard.

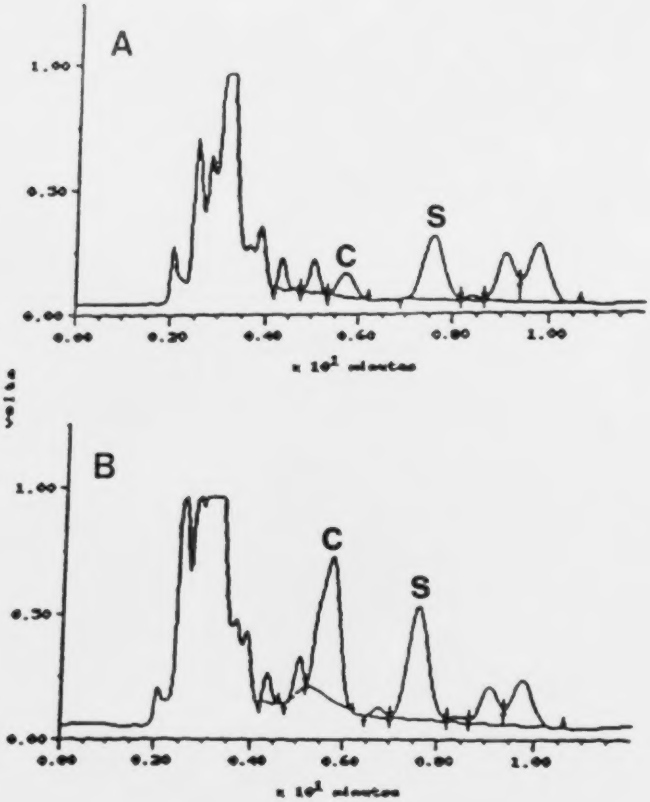

Figure 2.5 Representative chromatograms, obtained using a μ-Bondapak® C_{18} (10 μm) column, for plasma samples taken from a patient. (A) captopril administered alone; (B) captopril and moricizine administered simultaneously, showing interference with captopril peak. Peaks: c = captopril, s = internal standard.

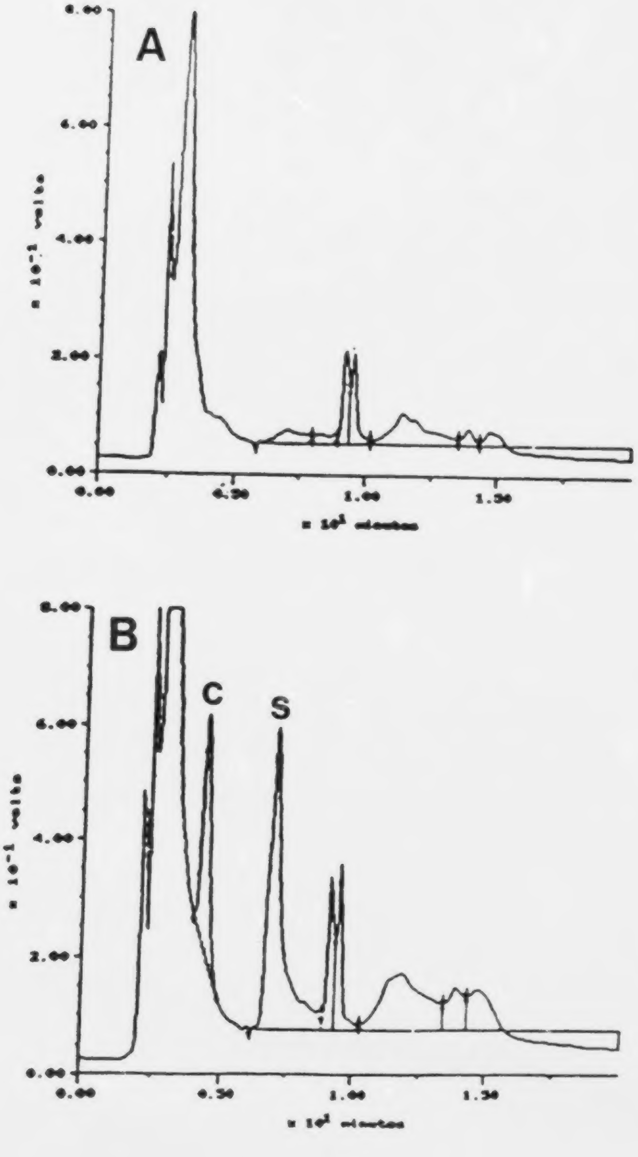

Figure 2.6 Representative chromatograms, obtained using a Zorbax® C_8 column, for plasma samples. (A) blank; (B) spiked with 600 ng/ml captopril. Peaks: c = captopril, s = internal standard.

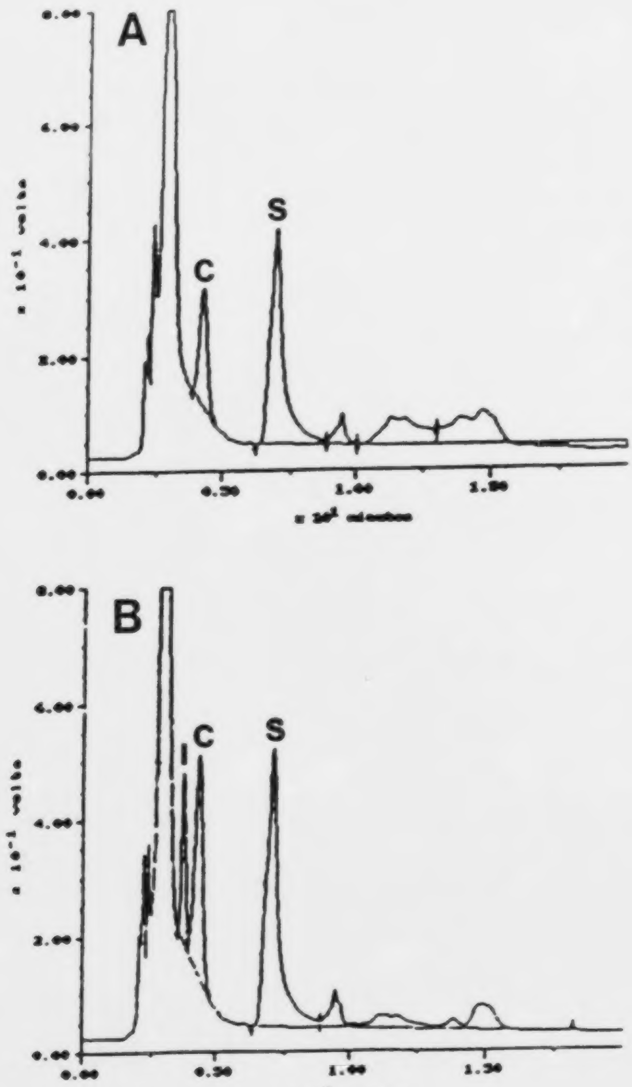

Figure 2.7 Representative chromatograms, obtained using a Zorbax® C_8 column, for plasma samples taken from a patient. (A) captopril administered alone; (B) captopril and moricizine administered simultaneously, showing interference peak separated from captopril. Peaks: c = captopril, s = internal standard, i = interference peak.

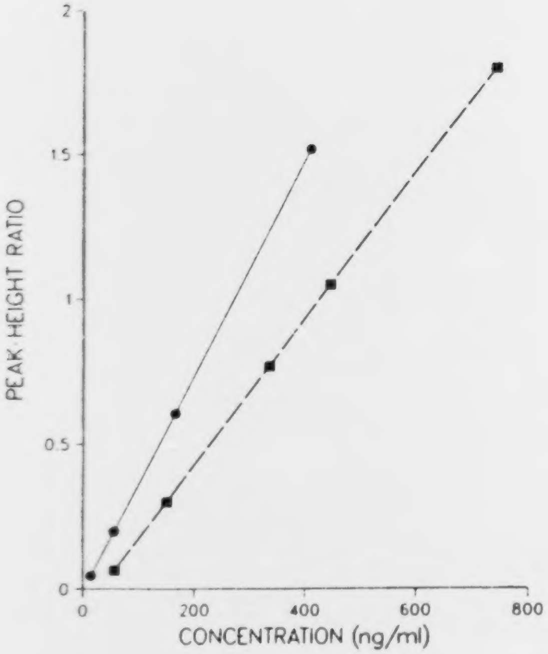

Figure 2.8 Calibration curves for unchanged (●) and total (■) captopril.

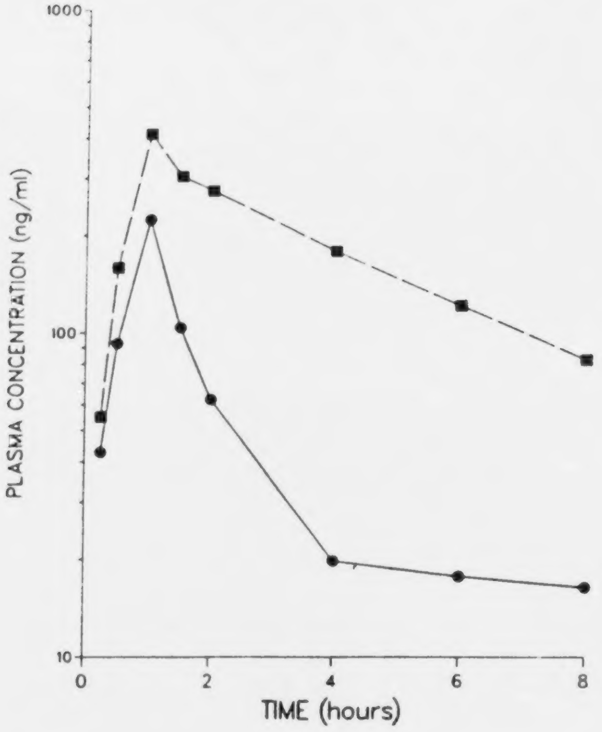

Figure 2.9 Plasma concentration versus time curves for unchanged (•) and total (▪) captopril after a 1 mg/kg oral dose to an infant.

2.6 References

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3. THE STABILITY OF CAPTOPRIL IN TAP WATER[2]

3.1 Introduction

The angiotensin-converting enzyme inhibitor, captopril, is currently being used to treat hypertension (*Mirkin et al. 1985*) and congestive heart failure in infants (*Montigny et al. 1989*). However, captopril is not commercially available in a liquid dosage form for use in these patients. It is common practice, therefore, to crush the tablets, dissolve the drug in water and administer an appropriate aliquot to the patient (*Mirkin et al. 1985, O'Dea et al. 1988*). This is inconvenient and costly as a fresh solution is prepared for each dose and much of the drug is wasted. It has been our experience that tap water is generally used to prepare solutions of captopril for oral use, both in hospitals and more so by outpatients, because it is more readily available than distilled water. Studies have been performed on the stability of captopril in Britton-Robinson buffer solutions at various pH levels, whole blood, plasma and urine (*Kawahara et al. 1981*), aqueous solution under controlled oxygen partial pressure with and without the addition of cupric ion (*Lee et al. 1987*), Japanese Pharmacopoeia 1st fluid (pH 1.2) and 2nd fluid (pH 6.8) (*Seta et al. 1988a*) and Japanese

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[2] A version of this chapter has been submitted for publication to the American Journal of Hospital Pharmacy.

Pharmacopoeia 2nd fluid in the presence of dog food, pancreatin or gall bladder powder and also in dog food supernatant with and without ascorbic acid (*Seta et al. 1988b*). While these studies show captopril to be relatively unstable in solution, information obtained from these studies is not directly applicable to the clinical situation. Hence this study was undertaken to determine the shelf-life of solutions of captopril in tap water.

3.2 Method

Capoten® 25 mg tablets (E.R. Squibb & Sons, Inc.) were crushed separately and each one added to 25 ml of tap water in individual volumetric flasks. This dilution is commonly used in the clinical situation because, assuming complete drug dissolution, it results in a drug concentration of 1 mg/ml which makes dosage calculation simple and at the same time allows a manageable volume of solution to be administered to the child. (A summary of the major chemical parameters for tap water in the City of Edmonton at the time of this study is shown in Table 3.1.) The flasks were shaken vigorously on a Vortex-Genie® (Fisher Scientific) at room temperature for 2 minutes. Five flasks each were incubated at 25, 50 and 75°C in a shaking water bath or refrigerated at 5°C. Samples were taken immediately after dissolving the drug and at intervals for up to 28 days. Samples (0.5 ml) were withdrawn from each flask at the times shown in Figure 3.1 and equilibrated to room temperature. Three 0.05 ml aliquots of each sample were each diluted with 1.2 ml disodium ethylenediamine tetraacetate solution (1 mM in water). 0.05 ml of each of these solutions was then added to 1 ml each of a 0.1 M phosphate buffer (pH 7) and unchanged captopril was quantitated using a high performance liquid chromatographic method developed in our laboratory (*Pereira et al. 1988*). No chromatographic interference was observed with this method because the only degradation product formed from captopril in water is a disulphide dimer (*Lee et al. 1987*) which is not detectable under the conditions of the assay for unchanged captopril.

Concentrations obtained were converted to percentages of the initial concentrations remaining. All chemicals used were of reagent grade.

Final drug concentrations at each temperature were compared with initial concentrations using the paired Student's t-test. Simple linear regression was used to fit the log percent captopril remaining versus time data. Degradation rate constants (K) were calculated from the slopes. Activation energy was estimated from the log K versus reciprocal of absolute temperature data according to the Arrhenius equation.

3.3 Results and Discussion

The mean (± SEM) concentration versus time data obtained, which show the degradation of captopril, are shown in Figure 3.1. Initial concentrations of captopril in solution indicated that dissolution of the drug was complete. Under the present experimental conditions, the degradation of captopril followed first order kinetics. Based on these data the times (± SEM) to reach 90 percent of the original captopril concentrations (i.e. the shelf-lives) at 75, 50 and 25°C were 2 ± 0.1, 4 ± 0.4 and 12 ± 1.2 days respectively. The degradation rate constants (± SEM) for captopril at 75, 50 and 25°C were 0.050 ± 0.003, 0.029 ± 0.003 and 0.0089 ± 0.0008 day^{-1} respectively. These constants were plotted against the reciprocal of the absolute temperature (Figure 3.2). The Arrhenius activation energy was thus estimated, using the Arrhenius equation, to be 7.1 kcal/mole.

Thus, captopril is more stable in tap water than in Britton-Robinson buffer at pH 7.9 (K=3.5 day^{-1}) or human whole blood (K=26.4 day^{-1}) at 24°C (*Kawahara et al. 1981*).

Concentration versus time data obtained at 5°C were not utilized because the variability in the measured concentrations and the relatively small magnitude of change resulted in the final concentrations being not significantly different from the initial concentrations, as determined by the paired t-test (p=0.05). (Final concentrations at 25, 50 and 75°C were all significantly different ($p<0.001$) from initial concentrations.) The Arrhenius plot (Figure 3.2) was therefore extrapolated and the degradation rate constant at 5°C was estimated to be 0.0041 day^{-1}. Based on this estimated K value, the shelf-life of a solution of captopril in tap water, at 5°C, was estimated to be 26 days.

3.4 Conclusion

We conclude that if a solution of captopril in tap water must be used, it may be stored for at least 3 weeks in a refrigerator (5°C) and need not be prepared freshly for each dose. It also appears feasible, from the point of view of the stability of captopril in water, for a reconstitutable liquid preparation of captopril to be introduced for pediatric use.

3.5 Table

Table 3.1 Summary of major chemical parameters for tap water in the City of Edmonton in 1988 as reported by the Rossdale Water Treatment Laboratory in Edmonton.

Parameters	1988 Average		
Aggressiveness Index*	11.6		
Alkalinity, Total	56	mg/L	(as $CaCO_3$)
Calcium	28	mg/L	
Hardness, Calcium	69	mg/L	(as $CaCO_3$)
Chloride	4.2	mg/L	
Chlorine, Total Residual	2.01	mg/L	
Copper	<0.01	mg/L	
Fluoride	0.99	mg/L	
Hardness, Total	116	mg/L	(as $CaCO_3$)
Iron	<0.03	mg/L	
Magnesium	11.8	mg/L	
Manganese	<0.01	mg/L	
Nitrate	<0.051	mg/L	(as N)
Nitrite	0.008	mg/L	(as N)
pH	8.0	units	
Phenols	<0.002	mg/L	
Potassium	0.82	mg/L	
Sodium	3.64	mg/L	
Sulphate	59.8	mg/L	
Sulphide	<0.05	mg/L	(as H_2S)
Total Dissolved Solids	160	mg/L	
Total Organic Carbon	3.0	mg/L	
Trihalomethanes	<0.001	mg/L	

* Aggressiveness Index = pH + $\log_{10}$ (AH), where A = total alkalinity and H = calcium hardness

3.6 Figures

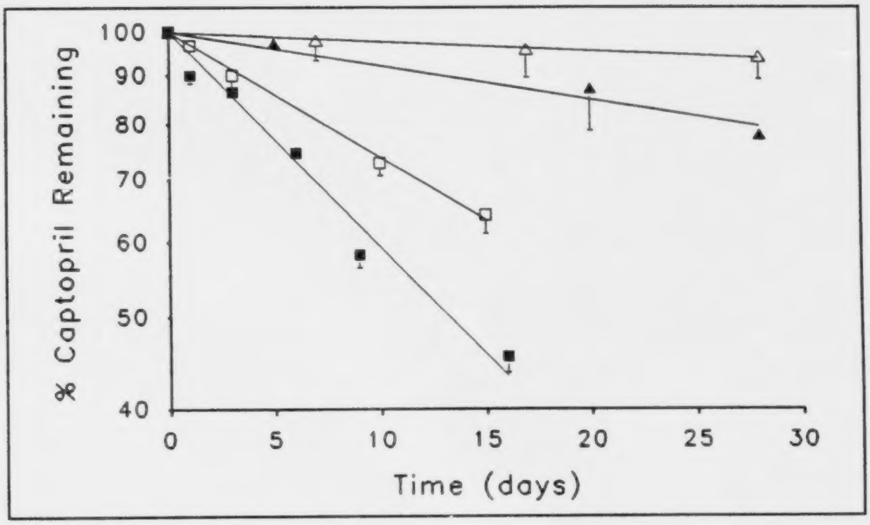

Figure 3.1 Log % captopril remaining in tap water versus time at 5°C (△), 25°C (▲), 50°C (□) and 75°C (■). (Values shown for % captopril remaining are means ± SEM of 5 tablets each, determined in triplicate.)

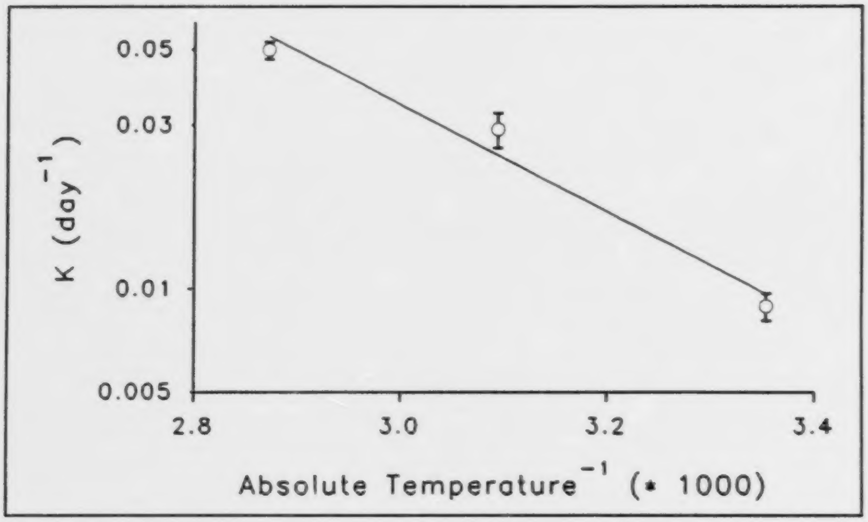

Figure 3.2 Arrhenius plot for captopril in tap water. (K values shown are means ± SEM determined from 5 tablets each.)

3.7 References

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4. THE PHARMACOKINETICS OF CAPTOPRIL IN INFANTS WITH CONGESTIVE HEART FAILURE[3]

4.1 Introduction

The angiotensin-converting enzyme inhibitor, captopril, is currently being used to treat hypertension (*Sinaiko et al. 1983, Mirkin et al. 1985*) and congestive heart failure (CHF) (*Scammell et al. 1987, Montigny et al. 1989*) in infants. However, the use of captopril in this patient population has been empirical because relevant kinetic data are unavailable. The kinetics of unchanged captopril were studied in adolescents (11-20 years old) with renal disease (*Sinaiko et al. 1983*). The clearance of captopril observed in these subjects was higher than that reported for adults with renal failure (*Duchin et al. 1984*). However these data may not be applicable to infants with CHF. Other studies (*Montigny et al. 1989, Shaddy et al. 1988*) provide hemodynamic data in infants with CHF treated with captopril, but in the absence of kinetic data, the relationship between drug concentration and effect is unknown. The objectives of this study are, therefore, to determine and evaluate the pharmacokinetics of captopril following oral dosing in infants under 2 years of age with CHF, and to determine the acute hemodynamic effects of captopril in these subjects. In this chapter special emphasis is placed on the pharmacokinetics of captopril in infants with CHF.

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[3] A version of this chapter has been published. Pereira CM, Tam YK, Collins-Nakai RL. *Ther Drug Monit* 1991;**13**:209-214.

4.2 Patients and Methods

Infants under 2 years of age, admitted to the University of Alberta Hospital, between 1986 and 1989, with CHF as a result of shunts at atrial, ventricular, or great vessel levels, or with idiopathic left ventricular dysfunction in whom cardiac catheterization was clinically indicated, were enroled. The study was approved by the University of Alberta Faculty of Medicine Ethics Review Committee. Informed consent was obtained from the parents of each child prior to enrolment.

Ten patients, 3 boys and 7 girls, were studied. The average age of these patients was 6.8 ± 4.6 months (range 2-15 months) and their average weight was 5.9 ± 1.8 kg (range 3.8-8.8 kg). The captopril dose was 1 mg/kg orally. Congestive heart failure was the result of atrial or ventricular septal defects in 7 patients and dilated cardiomyopathy in 3. Three of the 7 patients with septal defects were diagnosed additionally to have Down's syndrome, 2 to have patent ductus arteriosus and 1 to have bacterial endocarditis. This information is summarized in Table 4.1.

Diuretics were discontinued the day before the study.

In 4 patients they had to be restarted on the second day of the study for clinical reasons. Treatment with digoxin was continued throughout the study. Concurrent medications are listed in Table 4.1. Captopril (1 mg/kg/dose) was administered orally every 8 hours for 1 week. Aqueous solutions of captopril were freshly prepared from Capoten® tablets for each dose. Preliminary studies in our laboratory have shown that aqueous solutions of captopril are stable for the time required to prepare and administer the dose.

All patients received a pre-catheterization sedative mixture consisting of chlorpromazine, promethazine and chloral hydrate. The first dose of captopril was measured using a syringe (Luer Lok®, Becton Dickinson) and administered, during the catheterization procedure, *via* a nasogastric tube. All patients were fasted for 4 hours prior to the first dose of captopril. Blood samples of 2 mL each were withdrawn, at 0.0, 0.25, 0.5, 1.0, 1.5, 2.0, 4.0, 6.0 and 8.0 hours after the first dose of captopril and also before the first dose on days 3, 5 and 7, for the determination of plasma unchanged and total captopril levels. These levels were determined using the HPLC method developed in our laboratory (*Pereira et al. 1988*).

Resting oxygen consumption, plasma renin activity, heart rate (HR) and blood pressure (BP) and an echocardiogram were measured within 1 hour before the first dose of captopril.

Also, by means of cardiac catheterization, pressure (mmHg) in the right atrium (RA), pulmonary artery (PA), pulmonary wedge and/or the left atrium and aorta (Ao) and the oxygen saturation (%) in the Ao, RA, superior vena cava and PA were measured. These measurements were used to calculate pulmonary blood flow (L/min), cardiac output (L/min) and pulmonary and systemic resistances (mmHg/L/min/m^2) using standard methods (*Guyton 1984*).

The above measurements, including an echocardiogram, were repeated 60 minutes after the first dose of captopril.

Pharmacokinetic parameters were calculated from the plasma concentration versus time data by non-compartmental analysis using a computer program, LAGRAN (*Rocci et al. 1983*), assuming no lag time for drug absorption. Oral clearance, Cl_o, was estimated using the following equation:

$$Cl_o = \frac{Cl}{F} = \frac{D}{AUC_{0-\infty}}$$

Where Cl is the total body clearance, F is the bioavailability, D is the oral dose and $AUC_{0-\infty}$ is the total area under the plasma concentration versus time curve. The paired t-test was used to compare the clinical, hemodynamic and echocardiographic measurements made before and 1 hour after the administration of the first dose of captopril. The unpaired t-test was used to compare trough plasma captopril levels on day 1 with trough levels on days 3, 5 and 7 separately. A p value of less than 0.05 was considered to indicate a statistically significant difference.

4.3 Results

A pilot study was conducted, in which 4 infants received captopril, 0.3 mg/kg orally. This is the standard captopril dose used for the treatment of hypertension in children. No significant hemodynamic effects were seen and unchanged captopril was not detectable in the plasma of these infants, after the initial dose. The reasons for this observation are unknown. However, incomplete swallowing of the administered dose by the sedated infants may have been a contributing factor. The dose was therefore increased to 1 mg/kg and the first dose to each child was administered *via* a nasogastric tube. The higher dose, which is within the acceptable dosage range recommended by other investigators (*Sinaiko et al. 1983*), was safe and produced clinically effective plasma levels of captopril in the infants studied.

The plasma concentration (mean ± SD) versus time profiles of unchanged and total captopril after a 1 mg/kg oral captopril dose in the 10 study subjects are shown in Figure 4.1. The pharmacokinetic parameters calculated after the initial 1 mg/kg dose to these 10 patients are summarized in Table 4.2 for unchanged and Table 4.3 for total captopril.

The C_{max} for unchanged captopril in our subjects was 350 ± 184 ng/ml (range 116-732 ng/ml), T_{max} was 1.6 ± 0.4 h (range 1.0-2.0 h), the area under the plasma concentration versus time curve, AUC_{0-8}, was 912 ± 364 ng.h/ml (range 355-1494 ng.h/ml) and the estimated $AUC_{0-\infty}$ was 1019 ± 331 ng.h/ml (range 480-1512 ng.h/ml) after a 1 mg/kg oral captopril dose. The $t_{1/2}$ was 3.3 ± 3.3 h (range 1.2-12.4 h) and the mean residence time after an oral dose, MRT_o was 4.5 ± 3.0 h (range 1.9-12.9 h). Cl_o for unchanged captopril was estimated to be 1.1 ± 0.4 L/h/kg (range 0.7-2.1 L/h/kg). For total captopril, the C_{max} was 1088 ± 621 ng/ml (range 324-2531 ng/ml), the T_{max} was 2.7 ± 1.1 h (range 1.0-4.0 h), the $t_{1/2}$ was 3.4 ± 1.0 h (range 2.1-5.5 h), the MRT_o was 6.3 ± 1.2 h (range 5.0-8.6 h), the AUC_{0-8} was 5320 ± 3329 ng.h/ml (range 1451-12410 ng.h/ml) and the estimated $AUC_{0-\infty}$ was 7222 ± 4652 ng.h/ml (range 1865-16522 ng.h/ml). Total captopril levels were higher and showed a longer median half-life than unchanged captopril.

Trough levels of unchanged and total captopril on days 3, 5 and 7 of the study were not significantly different from levels 8 hours after the first dose [25 ± 14 ng/ml on day 1 (n=10) versus 14 ± 3 ng/ml on day 3 (n=4), 13 ± 4 ng/ml on day 5 (n=3) and 14 ± 2 ng/ml on day 7 (n=4) for unchanged captopril and 382 ± 268 ng/ml on day 1 (n=10) versus 227 ± 220 ng/ml on day 3 (n=8), 221 ± 172 ng/ml on day 5 (n=8) and

212 ± 171 ng/ml on day 7 (n=6) for total captopril; unchanged captopril concentrations were below the quantitation limit in three patients on day 3, two on day 5, and two on day 7 of the study.]

One hour after the initial dose of captopril, significant decreases were observed in heart rate (-7 ± 5 beats/minute; p=0.002), respiratory rate (-17 ± 8 breaths/minute; p=0.0002), pulmonary artery mean pressure (-13 ± 9 mmHg; p=0.001), mean arterial pressure (-14 ± 5 mmHg; p<0.0001), systemic resistance (-2 ± 2 mmHg/L/min/m^2; p=0.02) and pulmonary resistance (-1 ± 1 mmHg/L/min/m^2; p=0.02) (Figure 4.2). Plasma renin activity, measured in 8 subjects, did not change significantly. Echocardiography demonstrated significant decreases in left ventricular diastolic dimension (-2 ± 2 mm; p=0.03) and in left ventricular ejection time (-9 ± 9 milliseconds; p=0.02) and a significant increase in left ventricular pre-ejection period (10 ± 8 milliseconds; p=0.02) (Figure 4.3). There were no significant changes in left ventricular systolic dimension, shortening fraction and right ventricular systolic time intervals.

4.4 Discussion

In CHF, vascular perfusion to one or more parts of the body may be diminished. The pharmacokinetics of drugs may therefore be altered since blood flow may influence drug absorption, distribution and elimination (*Rowland et al. 1989*). The degree to which drug kinetics are affected varies depending upon the degree of congestive cardiac failure. Also, infants are continuously undergoing maturational changes in the physiological functions affecting drug disposition. Varying developmental stages and degrees of cardiac failure may serve, in part, to explain the variability in the pharmacokinetic parameters determined in our subjects. Unfortunately, there are no other pharmacokinetic data available (for infants) with which we may compare our results.

The half-life of unchanged captopril, after a 1 mg/kg oral dose was 3.3 ± 3.3 h and was within the range reported for adults with CHF i.e. 3.36 h reported by Rademaker et al. (*1986*) after a bolus intravenous captopril dose, 1.06 h reported by Cody et al. (*1982*) and 7 h reported by Shaw et al. (*1985*), each after a 25 mg oral captopril dose. Dose corrected (to 1 mg/kg) AUC and C_{max} values were also similar to those reported by Cody et al. (*1982*) (747 ng.h/ml and 366 ng/ml respectively) and by Shaw et al. (*1985*) (1056 ng.h/ml and 705 ng/ml respectively). The mean T_{max} (1.6 h) in our subjects was slightly longer than reported by Cody et al. (1.43 h) and by Shaw et al. (0.75 h).

We were unable to detect significant accumulation of unchanged or total captopril in plasma, on days 3, 5 and 7, partly due to the variability in the trough captopril levels and also due to the short $t_{1/2}$ of captopril, relative to the dosing interval. Correlation of plasma captopril levels with clinical or hemodynamic effect could not be accomplished because it was not feasible to obtain continuous or serial hemodynamic data in our subjects. We chose to measure the hemodynamic changes at 1 hour post-dose because this is approximately the time at which the acute changes are expected to be maximal, based on information in the literature (*Cody et al. 1982*). Also, at this time the infants are still sedated and the changes seen are unlikely to be due to external stimuli. Once the infants leave the cardiac catheterization laboratory, and are no longer sedated, various stimuli such as sudden noises, pain, fear, hunger and so on, would lead to changes in respiratory rate, blood pressure and heart rate. These changes are neither predictable nor controllable (as they may be in adults) and they would obscure drug effects. This may be part of the reason that to date no continuous or serial acute (0-8 h) hemodynamic data, in infants, after captopril administration, have been published.

Intense diuretic therapy is said to potentiate the renal response to captopril (*Dzau et al. 1985*). However, since diuretics were discontinued the day before the study, it is unlikely that the pharmacokinetics of the first dose of captopril were affected. There is, at present, no information that would lead us to suspect interaction between captopril and any of the components of the pre-catheterization sedative mixture or the antibiotics used. All infants were on digoxin therapy throughout the study. The combination of digoxin and captopril, given intravenously to adults with CHF, has been found to be synergistic (*Gheorghiade et al. 1989*). However, when baseline hemodynamic measurements were made, digoxin was at steady state. Also, the hemodynamic changes seen were qualitatively similar to changes reported after administration of captopril alone, to adults with CHF (*Shaw et al. 1985*). Hence, acute hemodynamic changes seen after the administration of captopril were attributed to captopril. These changes include significant decreases in pulmonary and systemic pressure and resistance. Also, echocardiographic results demonstrate decreases in left ventricular diastolic dimension and ejection time, suggesting better left ventricular function. Our data therefore suggest that, at least acutely, captopril has salutary effects on infants with CHF.

We conclude that the pharmacokinetic parameters for captopril in infants with CHF appear to be within the range previously reported for adults with CHF. Also, based on hemodynamic measurements made 1 hour after the first dose, the acute effects of captopril in infants with CHF, appear to be beneficial.

4.5 Tables

Table 4.1 Patient clinical data and concurrent medication.

Patient #	Age (m)	Weight (kg)	Sex	Diagnosis	Concurrent Medication
1.	15	8.8	M	CCM	D,Ms
2.	3	6.4	M	CCM	D
3.	12	8.6	F	CCM	D
4.	2	3.8	F	AVSD,BE	D,V,C,G,(F)
5.	6	6.0	F	VSD	D,(F)
6.	2	4.0	F	VSD	D,(F),A
7.	5	4.5	F	VSD,PDA,DS	D,(F)
8.	6	5.3	F	AVSD,PDA,DS	D
9.	12	7.4	M	VSD	D
10.	5	4.6	F	ASD,VSD,DS	D
Mean	6.8	5.9			
SD	4.6	1.8			

M, male; F, female; CCM, congestive cardiomyopathy; AVSD, atrioventricular septal defect; BE, bacterial endocarditis; VSD, ventricular septal defect; PDA, patent ductus arteriosus; DS, Down's syndrome; ASD, atrial septal defect; D, digoxin; Ms, mycostatin; V, vancomycin; C, cloxacillin; G, gentamycin; (F), furosemide restarted on second day of study; A, spironolactone + hydrochlorothiazide

Table 4.2 Pharmacokinetic parameters for unchanged captopril after a 1 mg/kg po captopril dose to 10 infants.

Patient #	C_{max} ng/ml	T_{max} h	$t_{½}$ h	MRT_o h	AUC_{0-8} ng.h/ml	$AUC_{0-\infty}$ ng.h/ml	Cl_o L/h/kg
1.	224	1.0	12.4	12.9	355	642	1.6
2.	164	1.8	2.8	4.8	690	851	1.2
3.	116	2.0	5.8	5.7	372	480	2.1
4.	332	1.5	2.0	3.6	1154	1262	0.8
5.	269	1.5	1.3	2.7	646	664	1.5
6.	732	1.0	1.5	1.9	937	962	1.0
7.	256	2.0	2.3	4.1	1040	1189	0.8
8.	535	1.5	1.2	2.5	1494	1512	0.7
9.	323	2.0	2.1	3.7	1188	1313	0.8
10.	544	1.5	1.6	3.2	1246	1315	0.8
Mean	350	1.6	3.3	4.5	912	1019	1.1
SD	184	0.4	3.3	3.0	364	331	0.4
Median	327	1.5	2.0	3.6	989	1076	0.9

Table 4.3 Pharmacokinetic parameters for total captopril after a 1 mg/kg po captopril dose to 10 infants.

Patient #	C_{max} ng/ml	T_{max} h	$t_{½}$ h	MRT_o h	AUC_{0-8} ng.h/ml	$AUC_{0-\infty}$ ng.h/ml
1.	412	1.0	3.5	5.5	1451	1865
2.	609	4.0	2.1	5.2	3047	3537
3.	324	1.5	5.5	8.6	1637	2678
4.	888	2.0	4.4	7.7	3531	5573
5.	791	2.0	2.6	4.9	3137	3725
6.	2531	2.0	3.6	5.9	12410	16522
7.	1283	4.0	2.9	6.5	5849	7945
8.	1261	2.0	2.4	5.0	6539	7898
9.	1146	4.0	3.0	5.8	6242	8168
10.	1636	4.0	4.1	7.6	9357	14308
Mean	1088	2.7	3.4	6.3	5320	7222
SD	621	1.1	1.0	1.2	3329	4652
Median	1017	2.0	3.3	5.9	4690	6736

4.6 Figures

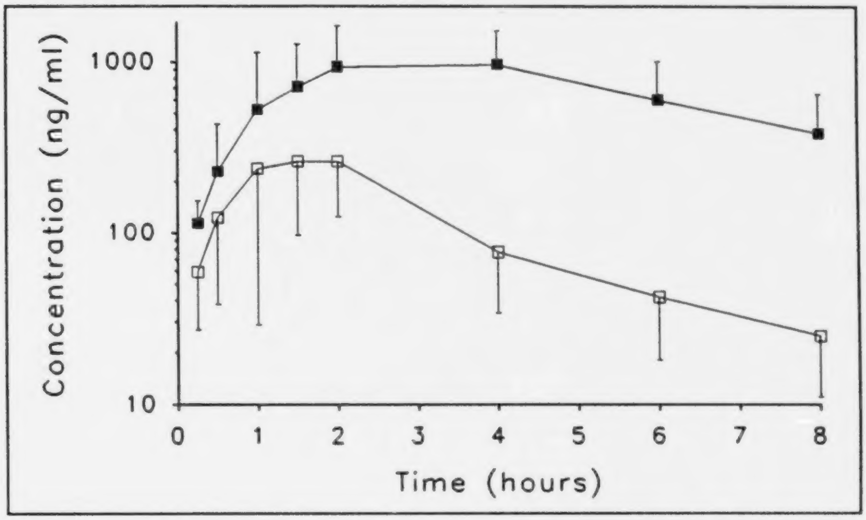

Figure 4.1 Plasma concentration versus time profiles for unchanged (□) and total (■) captopril after a 1 mg/kg po captopril dose to 10 infants (means ± SD shown).

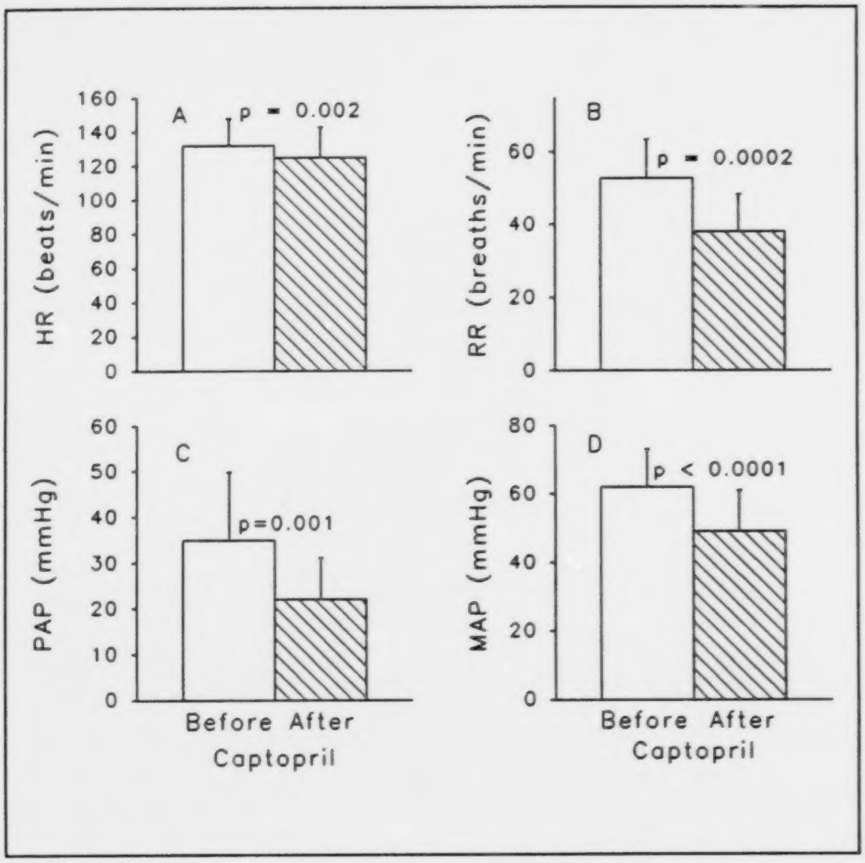

Figure 4.2 Heart (A) and respiratory (B) rates and mean pulmonary (C) and systemic (D) arterial pressures measured in 10 infants, before and 1 h after a 1 mg/kg po dose of captopril (means ± SD shown).

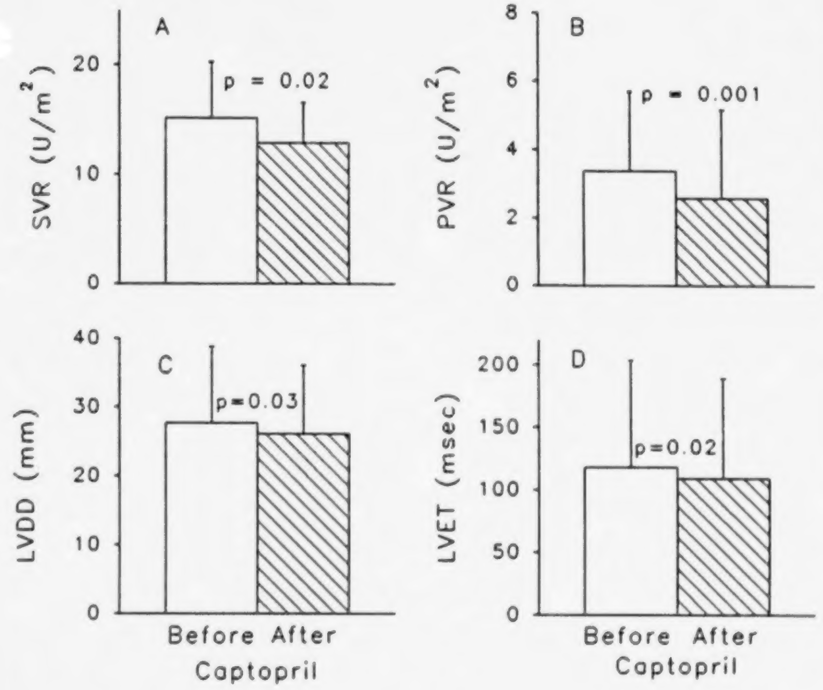

Figure 4.3 Systemic (A) and pulmonary (B) vascular resistances and left ventricular diastolic dimension (C) and ejection time (D) measured in 10 infants, before and 1 h after a 1 mg/kg po dose of captopril (means ± SD shown).

4.7 References

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5. PHARMACOKINETIC-PHARMACODYNAMIC MODELLING FOR CAPTOPRIL IN HEALTHY ANAESTHETIZED PIGLETS: APPLICATION TO HUMANS[4]

5.1 Introduction

The angiotensin converting enzyme (ACE) inhibitor, captopril, has been used in the treatment of hypertension and congestive heart failure (CHF) in children (*Mirkin et al. 1985, Montigny et al. 1989*) and in adults (*Cody et al. 1982*). However, the use of captopril has been empirical because a clear relationship between its pharmacokinetics and its pharmacodynamics has not, hitherto, been elucidated. Al-Furaih et al. (*1991*) report a linear relationship between the initial rise of plasma unchanged captopril concentration and the rise of plasma renin activity after the administration of 25 mg captopril orally to healthy volunteers. However plasma renin activity peaked later and the increase in activity was maintained longer than plasma concentrations of unchanged captopril. Shaw et al. (*1985*) did not observe any correlation between peak plasma unchanged captopril concentration and maximal reduction in systemic vascular resistance after administration of 25 mg captopril orally to patients with congestive heart failure. Richer et al. (*1984*) suggest that monitoring of plasma levels of unchanged captopril does not help to predict captopril's blood pressure lowering effects in hypertensive patients since they found no correlation between the relative bioavailability of captopril and the induced reduction in diastolic blood pressure after oral administration of captopril.

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[4] A version of this chapter has been submitted for publication in *J Cardiovasc Pharmacol* (1991)

In order to study the relationship between the pharmacokinetics and pharmacodynamics of captopril, it is necessary to have pharmacokinetic data and relevant serial pharmacodynamic data. Such data are not presently available for paediatric patients. Some kinetic-dynamic studies have been conducted on healthy adult subjects (*Al-Furaih et al. 1991*), patients with CHF (*Rademaker et al. 1986*, *Shaw et al. 1985*), hypertension (*Giudicelli et al. 1987*) and on patients undergoing peritoneal dialysis (*Fujimura et al. 1986*). However, no clear definition of the relationship between the pharmacokinetics and pharmacodynamics of captopril has emerged.

In an earlier study we conducted on the pharmacokinetics of captopril in infants with congestive heart failure (*Pereira et al. 1991*) it was not feasible to obtain serial hemodynamic data. Although all the infants were undergoing cardiac catheterization at the time of the first dose of captopril, the pharmacodynamic data obtained were not sufficient to allow modelling of the concentration-effect relationship. Since no kinetic-dynamic information for captopril in infants is available in the literature, an appropriate animal model was deemed necessary. Such a model would provide data suitable for an initial study of kinetic-dynamic relationships. Insight gained from the animal model could then be tested by application to data obtained in humans.

Swine have long been used in cardiovascular research (*Detweiler et al. 1966*) because of their anatomic and physiologic similarities to humans (*Bustad 1966*). It has been reported that the first 2.5 weeks of a piglet's life correspond to the first 6 months of the human infant's life (*Boudreaux et al. 1984*). Experimentally induced cardiac failure in swine has also been described (*Maaske et al. 1966, Lumb et al. 1966*). The anaesthetized piglet was chosen in the hope that it would mimic the acute studies in sedated infants undergoing cardiac catheterization. The objectives of this study are, therefore, to evaluate the piglet as an appropriate animal model in which to study the pharmacokinetics and pharmacodynamics of captopril. This includes the application of knowledge gained about the relationship between the pharmacokinetics and pharmacodynamics of captopril to literature data from studies on human subjects.

Porcine models for chronic congestive heart failure have been reported (*Maaske et al. 1966, Lumb et al. 1966*); however the resources necessary for the use of this model were not available for the present investigation. Myocardial infarction (MI), on the other hand, is acutely inducible and it was speculated that changes in the pharmacokinetics and/or pharmacodynamics of captopril due to myocardial dysfunction, thus induced, may also occur in congestive heart failure. Also, in addition to use in the treatment of hypertension and congestive heart failure, captopril is presently being recommended for use in adults with MI (*Ambrosioni et al. 1989*). However, no information is available on the potential effects of captopril in children with MI (for example, in Kawasaki syndrome). It is our intention therefore to conduct a pilot study to determine the effects of captopril in piglets with surgically induced MI as this may provide some insight into the potential effects of captopril on children with MI.

5.2 Methods

Five healthy piglets, of the Yorkshire strain, were studied. The average age of the animals was 12 ± 1.6 days (range 10 - 14 days) and their average weight was 4 ± 0.2 kg (range 3.8 - 4.2 kg). The animals were surgically instrumented as previously described (*Coe et al. 1897*) for a chronic preparation, with the exception that only one electromagnetic flow probe was used (around the main pulmonary artery). Also, the animals were studied acutely following instrumentation, while anaesthetized. In brief, general anaesthesia was induced and maintained with oxygen (4 L/min), nitrous oxide (4 L/min) and halothane (1.2% for induction and 0.75% for maintenance of anaesthesia). An electromagnetic flow probe was placed around the main pulmonary artery. Catheters were placed in the main pulmonary artery, aorta, left ventricle, and right and left atria. A Mikro-Tip® hi-fi catheter (Millar Instruments, Inc., USA) was used to measure the rate of change of left ventricular pressure, LV dP/dt, a measure of myocardial contractility. The ductus arteriosus was ligated. After the surgery was completed the animals were allowed to stabilize for approximately 0.5 h. The animals were considered to be in a hemodynamically stable condition if three consecutive measurements of aortic pressure taken approximately 5 min apart were within 5% of each other. Captopril (E.R. Squibb & Sons, Inc.) was dissolved in sterile normal saline and 0.2 mg/kg was administered as an intravenous (iv) bolus. Blood samples of 1 ml each were collected before and at 0.33, 5, 10, 15, 20, 30, 45, 60, 75, 90, 105, and 120 minutes after the dose for the determination of concentrations of unchanged captopril in plasma. Preliminary experiments showed that more than 90% of the area under the plasma concentration versus time curve for unchanged captopril would be accounted for within 2 h after an iv bolus dose to healthy anaesthetized piglets. Plasma captopril concentrations were measured using the HPLC method developed in our laboratory (*Pereira et al. 1988*). Heart rate, main pulmonary artery flow, LV dP/dt and pressures in the right and left atria, pulmonary artery, aorta and left ventricle were also measured at the intervals mentioned above. A sham experiment was also performed in order to confirm the stability of the preparation over 2 h. The above procedures were followed except that sterile normal saline was injected instead of the captopril solution.

In addition to the healthy animals, 6 piglets, aged 14 ± 1.6 days (range 12 - 16 days), weighing 3 ± 0.4 kg (range 2.5 - 3.6 kg) were acutely studied after induction of MI. The experiments were conducted as for the healthy animals except that in addition, a small apical infarct was produced by ligation of a short segment of the left anterior descending coronary artery 15 minutes before the administration of captopril. A sham experiment was also performed to confirm that the piglets would stabilize and remain stable for at least 2 h, after the production of an infarct. Piglets that went into ventricular arrhythmia as a consequence of ischemia were excluded.

Pressures, heart rate, main pulmonary artery flow (cardiac output) and LV dP/dt were determined from the recorder tracings as shown in Figure 5.1.

For the healthy animals, the paired t-test was used to determine the significance of the maximum percent change of the hemodynamic parameters from baseline. A p value of less than 0.05 was considered to indicate a statistically significant difference.

Pharmacokinetic parameters were calculated from the plasma concentration versus time data by non-compartmental analysis using a computer program, LAGRAN (*Rocci et al. 1983*). Aortic pressure and heart rate versus time profiles were modelled using an effect-compartment model (*Sheiner et al. 1979*) combined with a linear pharmacodynamic model (*Holford et al. 1981*). Equations for this model have been previously derived (*Holford et al. 1981*). The following equations represent the drug concentration, C_e, at the hypothetical effect site:

a) with a 2-compartment pharmacokinetic model after an iv bolus dose:

$$C_e = \frac{DK_{e0}}{V_c}\left[\frac{(K_{21}-\alpha)e^{-\alpha t}}{(\beta-\alpha)(K_{e0}-\alpha)} + \frac{(K_{21}-\beta)e^{-\beta t}}{(\alpha-\beta)(K_{e0}-\beta)} + \frac{(K_{21}-K_{e0})e^{-K_{e0}t}}{(\alpha-K_{e0})(\beta-K_{e0})}\right]$$

b) with a 2-compartment pharmacokinetic model after oral administration:

$$C_e = \frac{FDK_aK_{e0}}{V_c}\left[\frac{(K_{21}-\alpha)e^{-\alpha t}}{(\beta-\alpha)(K_a-\alpha)(K_{e0}-\alpha)} + \frac{(K_{21}-\beta)e^{-\beta t}}{(\alpha-\beta)(K_a-\beta)(K_{e0}-\beta)} + \frac{(K_{21}-K_a)e^{-K_a t}}{(\alpha-K_a)(\beta-K_a)(K_{e0}-K_a)} + \frac{(K_{21}-K_{e0})e^{-K_{e0} t}}{(\alpha-K_{e0})(\beta-K_{e0})(K_a-K_{e0})}\right]$$

c) with a 1-compartment pharmacokinetic model after oral administration:

$$C_e = \frac{FDK_aK_{e0}}{V_c}\left[\frac{e^{-Kt}}{(K_a-K)(K_{e0}-K)} + \frac{e^{-K_a t}}{(K-K_a)(K_{e0}-K_a)} + \frac{e^{-K_{e0} t}}{(K-K_{e0})(K_a-K_{e0})}\right]$$

where D is the dose, K_{e0} is the equilibration rate constant, V_c/F is the volume of distribution of the central compartment divided by bioavailability, α and β are exponential rate constants for a 2-compartment model, K_{21} is the transfer rate constant from compartment 2 to compartment 1, t is time after the dose, K_a is the absorption rate constant and K is the elimination rate constant for a 1-compartment open model. In all cases elimination was assumed to occur from the central compartment only. Also, the effect compartment was assumed to be connected to the central compartment.

The compartmental pharmacokinetic parameters necessary for this model were obtained using the computer program PCNONLIN (*Statistical Consultants, Inc., 1989*). A two-compartment open model with bolus input was used to describe the plasma concentration versus time data from the piglets and a one or two compartment open model with bolus or first order input, as appropriate, was used to describe concentration versus time data taken from the literature. The expression for C_e, with all parameters (except K_{e0}) entered as constants, was substituted into the following equation which represents a linear pharmacodynamic model which was used to describe observed effect versus time data:

$$E = (S * C_e) + E_0$$

where E is the effect being considered, S is the slope of the line relating concentration and effect and E_0 is the baseline effect.

The parameters K_{e0}, S and E_0 were estimated iteratively, using non-linear least squares regression analysis using PCNONLIN (*Statistical Consultants, Inc., 1989*).

The pharmacokinetic-pharmacodynamic model used to describe data obtained in the piglets was applied to literature data on human subjects. Literature data values were obtained from figures in published papers using a digitizer with the program SigmaScan (*Jandel Scientific, 1988*).

5.3 Results

Sham experiments (i.e. no captopril given) with one healthy piglet and one with induced MI confirmed the findings of other investigators (*Coe et al. 1987, Kingma et al. 1986, de Graeff et al. 1987*) in that these surgical preparations were found to be stable for at least 2 h. Values of hemodynamic parameters at baseline and at 15, 60 and 120 min for the healthy and MI sham experiments are shown in Table 5.1 and 5.2 respectively.

The plasma concentration (mean ± SD) versus time profile of unchanged captopril after a 0.2 mg/kg iv dose to 5 piglets is shown in Figure 5.2. The values of the non-compartmental pharmacokinetic parameters, calculated after a single dose, for unchanged captopril are summarized in Table 5.3.

The terminal elimination half-life, $t_{½}$, was estimated to be 0.8 ± 0.2 h (range 0.5 - 1.0 h) for unchanged captopril after a 0.2 mg/kg iv dose of captopril. Total body clearance, Cl_{TB}, was estimated to be 1.3 ± 0.2 L/kg/h (range 1.06 - 1.71 L/kg/h) and the volume of distribution at steady state, V_{dss}, was calculated to be 0.8 ± 0.1 L/kg (range 0.6 - 1.0 L/kg). The area under the plasma concentration versus time curve, AUC_{0-2}, was 144 ± 21 ng.h/ml (range 107 - 171 ng.h/ml) and the estimated $AUC_{0-\infty}$ was 160 ± 26 ng.h/ml (range 117 - 188 ng.h/ml).

In the healthy piglets, the maximum percent change (± SD) in hemodynamic parameters were as follows: significant decreases were observed in pulmonary artery pressure (-22 ± 13; p=0.008), aortic pressure (-42 ± 18; p=0.003), heart rate (-21 ± 11; p=0.01) and LV dP/dt (-46 ± 20; p=0.006). Changes in cardiac output (-25 ± 27) and systemic and pulmonary vascular resistances (-20 ± 31 and 42 ± 41 respectively) were not significant.

Aortic pressure (mean ± SD) versus time is shown in Figure 5.3 and heart rate in Figure 5.4. The solid line in each graph represents the model prediction for that parameter. The values of the compartmental pharmacokinetic model parameters (in the healthy piglets) used in the kinetic dynamic model are summarized in Table 5.4. The pharmacokinetic-pharmacodynamic model parameters describing the relationship between C_e and effect in the healthy piglets are summarized in Table 5.5 for aortic pressure and Table 5.6 for heart rate.

Four of the 6 piglets with induced MI died within 45 min after administration of a bolus dose of captopril, 0.2 mg/kg iv, as a result of the acute fall in systemic pressure and bradycardia followed by asystole. Two animals survived the duration of the 2 h experiment. The maximum percent changes in hemodynamic parameters (mean reported) in these animals were as follows: aortic pressure, -52, systemic vascular resistance, -50, pulmonary vascular resistance, +72 and LV dP/dt, -19.

The values of the compartmental pharmacokinetic model parameters and the parameter estimates for the pharmacokinetic-pharmacodynamic model when applied to literature data, are summarized in Table 5.7. The type of pharmacokinetic model used for each data set is also indicated in Table 5.7. The model was fitted to plasma renin activity and blood pressure versus time data obtained in healthy adults after administration of 25 mg captopril orally (*Al-Furaih et al. 1991*) (Figure 5.5) [Note that the data presented in Figure 5.5, B and C, were collected after 'sublingual' administration of captopril, i.e. the drug was retained in the mouth for 10 min and then swallowed with water. However, the plasma concentration versus time profiles presented showed no captopril in the plasma until after the drug was swallowed. Since a lag-time of 11 min was required to fit the concentration versus time data this was taken to be the time of drug administration. The route of administration was taken to be oral]; plasma renin activity and systemic vascular resistance versus time data after administration of 25 mg of captopril orally to patients with congestive heart failure (*Shaw et al. 1985*) (Figure 5.6);

systemic vascular resistance, cardiac output and blood pressure versus time data obtained after administration of captopril, 25 mg iv, to patients with congestive heart failure (*Rademaker et al. 1986*) (Figure 5.7); percent inhibition of plasma ACE activity versus time data in hypertensive patients after the administration of 50 mg of captopril orally (*Giudicelli et al. 1987*) (Figure 5.8) and mean blood pressure versus time data obtained after administration of 50 mg captopril orally to patients undergoing peritoneal dialysis (*Fujimura et al. 1986*) (Figure 5.9).

5.4 Discussion

Animal model: As seen in Tables 5.1 and 5.2, the maximum percent change in hemodynamic parameters, for both the healthy piglet and the animal with induced MI, over 2 h suggest that the surgical preparation is relatively stable over at least 2 h. Halothane has been shown to be a negative inotrope in newborn piglets (*Boudreaux et al. 1984*). However, baseline hemodynamic measurements were made before drug administration, while the animal was stable under anaesthesia. This implies that any further changes in hemodynamic parameters after the administration of captopril were indeed drug-induced and not due to surgical trauma or the anaesthetic agent. While it is possible that surgical trauma and/or the use of an anaesthetic agent may affect the intensity or indeed the nature of the response to the drug, the observed changes in aortic pressure were qualitatively similar to changes in blood pressure observed in humans, after captopril administration. Therefore a model developed to describe aortic pressure changes in piglets would also be applicable to data obtained in humans. The qualitative similarity in pressure response to captopril is illustrated in Figure 5.10 which compares the maximum percent change (mean shown) in aortic pressure versus time in the piglets with similar (published) data on humans (*Al-Furaih et al. 1991*, *Fujimura et al. 1986*, *Rademaker et al. 1986*).

Heart rate in the piglets decreased significantly and while the degree of the bradycardia may have been increased by halothane (*Boudreaux et al. 1984*), a significantly decreased heart rate was also observed in sedated infants (no halothane present) 1 h after receiving captopril, during cardiac catheterization (*Pereira et al. 1991*) and in adult hypertensive patients (*Sturani et al. 1982*). Changes in systemic and pulmonary vascular resistances and cardiac output were not significant.

In the piglets, $t_{1/2}$ was estimated to be 0.78 ± 0.16 h. Estimates for $t_{1/2}$ of unchanged captopril in healthy humans range from 0.35-1.9 h (*Onoyama et al. 1981*, *Duchin et al. 1982*). V_{dss} was calculated to be 0.81 ± 0.14 L/kg in the piglets and is reported to be 0.7-0.75 L/kg in healthy adult humans (*Duchin et al. 1982*, *Singhvi et al. 1982*). Cl_{TB} was 1.29 ± 0.24 L/h/kg in the piglets and is reported to be 0.8 ± 0.05 L/h/kg in healthy adults (*Singhvi et al. 1982*).

Thus, in general, the anaesthetized piglet is a viable model in which to study the relationship between the pharmacokinetics and pharmacodynamics of captopril.

The results of our pilot study in piglets with induced MI suggest that extreme caution should be exercised if captopril is to be used to manage children with MI. This is because in the piglets the bradycardia apparently induced by captopril was severe and when combined with the acute drop in systemic pressure and reduced contractility caused by myocardial ischaemia, the results were fatal in 4 out of 6 animals.

Pharmacokinetic-pharmacodynamic model: The effects of captopril are brought about by more than one mechanism. Primarily, inhibition of ACE in tissue and plasma leads to reduction of circulating levels of the potent endogenous vasoconstrictor angiotensin II. However, captopril's effects on aldosterone secretion, bradykinin degradation and on vasoactive prostaglandins must also be taken into account (*Brogden et al. 1988*). Since the drug has several modes of action and the observed effect may be a result of a cascade of biochemical events, it is not surprising that no direct relationship between plasma drug concentration and observed effect is seen and there is an 'equilibration delay' between peak plasma captopril concentrations and peak effect. Also, since the amount of drug at the site or sites of action may be negligible compared with the amount in plasma (*Sheiner et al. 1979*) drug effects may still be present when plasma drug concentrations are no longer detectable. Since it is not feasible to track the individual mechanisms by which captopril acts, the effect-compartment model proposed by Sheiner et al. (*1979*) is useful in that it considers only an 'equilibration delay', quantified by an equilibration rate constant K_{e0}, thereby taking the sum of all the individual mechanisms of action, known or unknown, into account. This model may be used to generate concentrations at a hypothetical effect site. These estimated concentrations may then be related to the observed effect using a simple pharmacodynamic model. The linear pharmacodynamic model is adequate when the observed effect does not approach the maximum possible effect (E_{max}). In cases where a maximum effect is reached, a different pharmacodynamic model such as the E_{max} model (*Holford et al. 1981*) may be used.

The effect-compartment model (with a 2-compartment pharmacokinetic model and iv bolus input) combined with a linear pharmacodynamic model fit aortic pressure and heart rate versus time data well. The correlation coefficients (observed data versus predicted values) for aortic pressure and heart rate versus time were 0.913 and 0.837 respectively.

The same model as above (with 1 or 2-compartment kinetic models and iv or oral input as appropriate) was also applied to 10 published data sets and resulted in good fits with correlation coefficients ranging from 0.848 to 0.992.

A clear relationship between the pharmacokinetics and pharmacodynamics of captopril has therefore been established wherein effect increases linearly with concentration at the effect site.

The model describes most of the commonly reported effect measurements for captopril well, however considerable variability was seen in the pharmacokinetic parameters for captopril in the piglets (Table 5.3 lists non-compartmental kinetic parameter estimates and Table 5.4 lists compartmental kinetic parameter estimates in individual piglets) as was seen in infants (*Pereira et al. 1991*). Hence the variability in the estimated parameters of the kinetic-dynamic model, was not unexpected (see Tables 5.5 and 5.6 for kinetic-dynamic parameter estimates in individual piglets). Now that a concentration-effect relationship has been established for captopril, considerably more and larger studies are required in order to determine whether each effect parameter is associated with unique pharmacokinetic-pharmacodynamic and pharmacodynamic model parameter (K_{e0}, slope) values and what these values are for various populations.

5.5 Conclusion

We conclude the following:

1. The anaesthetized piglet is a viable model in which to study the relationship between the pharmacokinetics and the pharmacodynamics of captopril.

2. A parametric pharmacokinetic-pharmacodynamic model combined with a linear effect model was used to define a relationship between the kinetics and dynamics of captopril. The parametric kinetic-dynamic model included an effect compartment connected to the central compartment of a multi-compartment kinetic model. The model fit data obtained in the piglets and also published data on humans, very well.

We have therefore established a clear relationship between the pharmacokinetics and pharmacodynamics of captopril. The linear pharmacodynamic model appears to fit most clinical situations.

3. Extreme caution is recommended if captopril is used to manage children with MI.

5.6 Tables

Table 5.1 Haemodynamic parameters in a sham experiment with a healthy piglet.

Time min	AoP mmHg	HR b/min	CO ml/min/kg	SVR U/kg	PVR U/kg	dP/dt mmHg/s
0	62	222	170	1276	185	1500
15	62	229	175	1240	180	1600
60	55	182	160	1203	197	1400
120	62	208	180	1206	175	1500

AoP, aortic pressure; HR, heart rate; CO, cardiac output; SVR, systemic vascular resistance; PVR, pulmonary vascular resistance; dP/dt, rate of change of left ventricular pressure

Table 5.2 Hemodynamic parameters in a sham experiment with a piglet with induced myocardial infarction.

Time min	Aop mmHg	HR b/min	CO ml/min/kg	SVR U/kg	PVR U/kg	dP/dt mmHg/s
0*	60	172	420	479	51	1000
15**	58	211	300	626	36	800
60	48	190	250	652	43	900
120	54	190	280	641	51	1000

AoP, aortic pressure; HR, heart rate; CO, cardiac output; SVR, systemic vascular resistance; PVR, pulmonary vascular resistance; dP/dt, rate of change of left ventricular pressure; *, pre-ligation; **, post-ligation

Table 5.3 Non-compartmental pharmacokinetic parameter estimates for unchanged captopril after a 0.2 mg/kg iv captopril dose to 5 piglets.

Piglet #	$t_{½}$ h	Cl_{TB} L/h/kg	V_{dss} L/kg	AUC_{0-2} ng.h/ml	$AUC_{0-\infty}$ ng.h/ml
1.	0.50	1.35	0.77	141.11	148.34
2.	0.82	1.06	0.64	171.65	188.26
3.	0.98	1.07	0.93	155.18	186.13
4.	0.84	1.71	0.99	107.10	116.76
5.	0.75	1.26	0.69	147.16	159.01
Mean	0.78	1.29	0.81	144.44	159.70
SD	0.16	0.24	0.14	21.30	26.41

Table 5.4 Compartmental pharmacokinetic parameter estimates for unchanged captopril after a 0.2 mg/kg iv captopril dose to 5 piglets (2-compartment open model with bolus input).

Piglet #	α (min^{-1})	β (min^{-1})	V_c (L/kg)	K_{21} (min^{-1})
1.	0.0700	0.0208	0.57	0.0366
2.	0.4018	0.0242	0.11	0.0577
3.	0.5592	0.0243	0.14	0.0941
4.	0.7141	0.0332	0.16	0.1160
5.	0.4206	0.0322	0.15	0.0884
Mean	0.4331	0.0270	0.23	0.0785
SD	0.2134	0.0049	0.17	0.0281

Table 5.5 Pharmacokinetic-pharmacodynamic model parameter estimates for aortic pressure after a 0.2 mg/kg iv captopril dose to 5 piglets.

Piglet	K_{e0} min^{-1}	S	E_0 mmHg	r
1.	0.01721	-0.41981	45.96	0.940
2.	0.00633	-0.36921	57.75	0.924
3.	0.00898	-0.58644	43.67	0.970
4.	0.00583	-0.32241	54.00	0.811
5.	0.01098	-0.30126	60.54	0.922
Mean	0.00987	-0.39983	52.38	0.913
SD	0.00412	0.10185	6.56	0.054

K_{e0}, equilibration rate constant; S, slope of line predicted by linear pharmacodynamic model; E_0, baseline effect; r, correlation coefficient (observed versus predicted values)

Table 5.6 Pharmacokinetic-pharmacodynamic model parameter estimates for heart rate after a 0.2 mg/kg iv captopril dose to 5 piglets.

Piglet	K_{e0} min^{-1}	S	E_0 beats/min	r
1.	0.03118	-0.42705	128.40	0.881
2.	0.01913	-0.31617	130.17	0.841
3.	0.00767	-0.78964	133.24	0.965
4.	0.02644	-0.11850	132.35	0.699
5.	0.00582	-0.64918	164.30	0.797
Mean	0.01805	-0.46011	137.69	0.837
SD	0.01002	-0.23784	13.41	0.088

K_{e0}, equilibration rate constant; S, slope of line predicted by linear pharmacodynamic model; E_0, baseline effect; r, correlation coefficient (observed versus predicted values)

Table 5.7 Compartmental pharmacokinetic model and kinetic-dynamic model parameter estimates for literature data.

Reference	Kinetic Model	V_c/F (L)	K_a (min^{-1})	K_{10} (min^{-1})	K_{21} (min^{-1})	α (min^{-1})	β (min^{-1})	Effect Parameter	K_{e0} min^{-1}	S	E_0	r
1	1C,po	74.3	0.0186	0.0122	na	na	na	PRA	0.04163	0.06138	2.72	0.992
	1C,po(sl)	88.3	0.0486	0.0129	na	na	na	PRA(sl)	0.03497	0.08882	3.08	0.981
								SBP(sl)	0.08239	-0.04363	105.37	0.848
2	2C,po	64.8	0.0447	na	0.0107	0.0212	0.0085	PRA	0.01903	0.13028	9.22	0.873
								SVR	0.02175	-3.62220	2113.06	0.880
3	2C,iv	14.1	na	na	0.0144	0.0754	0.0096	SVR	0.05103	-0.93042	1865.13	0.920
								CO	0.07685	0.00081	3.44	0.961
								MBP	0.03591	-0.02873	87.78	0.891
4	1C,po	79.6	0.0929	0.0087	na	na	na	PCEA	0.00493	0.51573	0.00	0.946
5	2C,po	84.2	0.0304	na	0.0121	0.0168	0.0078	MBP	0.07182	-0.06774	90.34	0.860

1, Al-Furaih et al. 1991; 2, Shaw et al. 1985; 3, Rademaker et al. 1986; 4, Giudicelli et al. 1987; 5, Fujimura et al. 1986; K_{e0}, equilibration rate constant; S, slope of line predicted by linear pharmacodynamic model; E_0, baseline effect; r, correlation coefficient (observed versus predicted values); PRA, plasma renin activity (ng/ml/h); SBP, systolic blood pressure (mmHg); sl, after 'sublingual' administration; SVR, systemic vascular resistance (dyn s cm^{-5}); CO, cardiac output (L/min); MBP, mean blood pressure (mmHg); PCEA, plasma angiotensin converting enzyme activity inhibition (%); 1C, 1-compartment open model; po, oral administration; 2C, 2-compartment open model; iv, intravenous administration; sl, 'sublingual' administration; na, not applicable

5.7 Figures

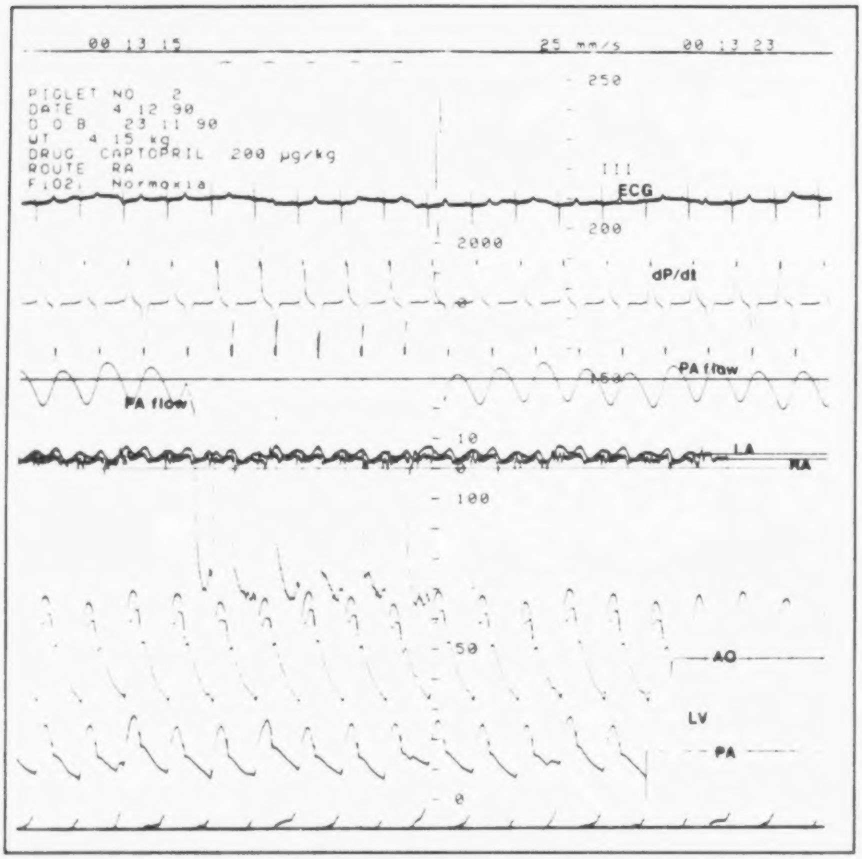

Figure 5.1 Pressure and flow tracings from a typical experiment with a healthy anaesthetized piglet. PA, pulmonary artery; LV, left ventricle; AO, aorta; RA, right atrium; LA, left atrium.

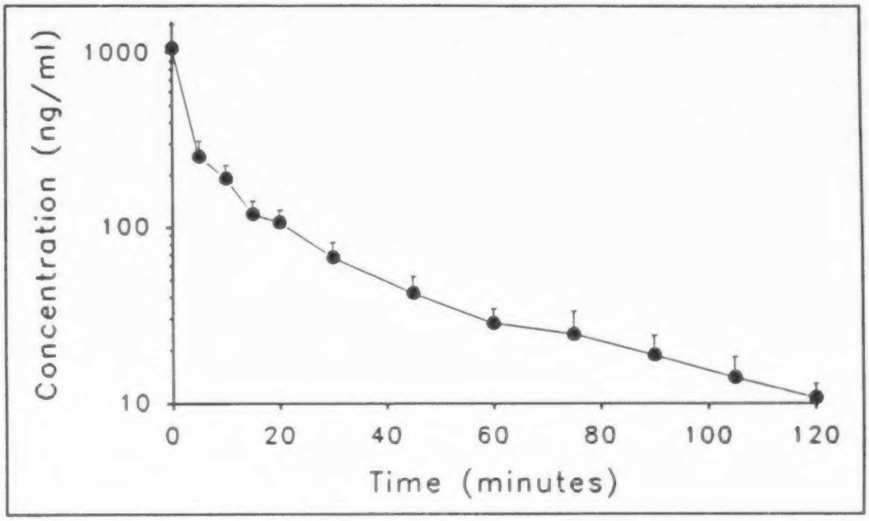

Figure 5.2 Plasma concentration versus time profile for captopril after a 0.2 mg/kg iv captopril dose to 5 piglets (means ± SD shown).

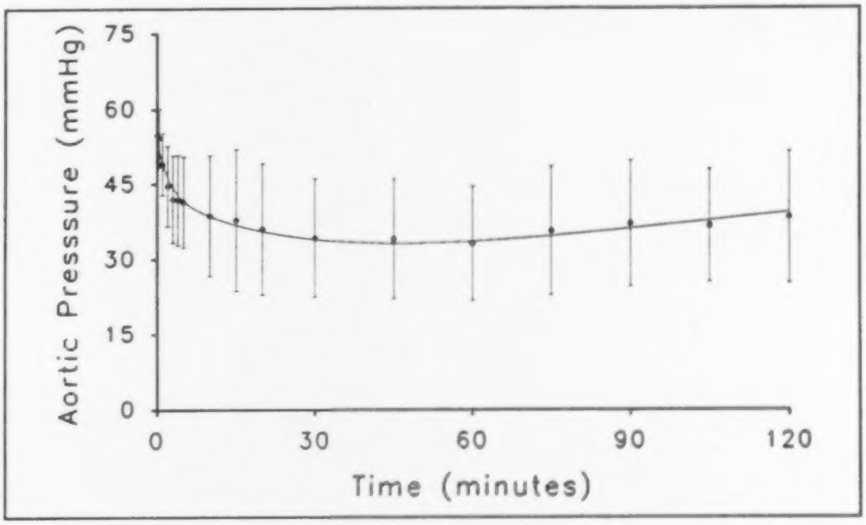

Figure 5.3 Aortic pressure versus time profile for captopril after a 0.2 mg/kg iv captopril dose to 5 piglets (means ± SD shown). Solid line represents model prediction.

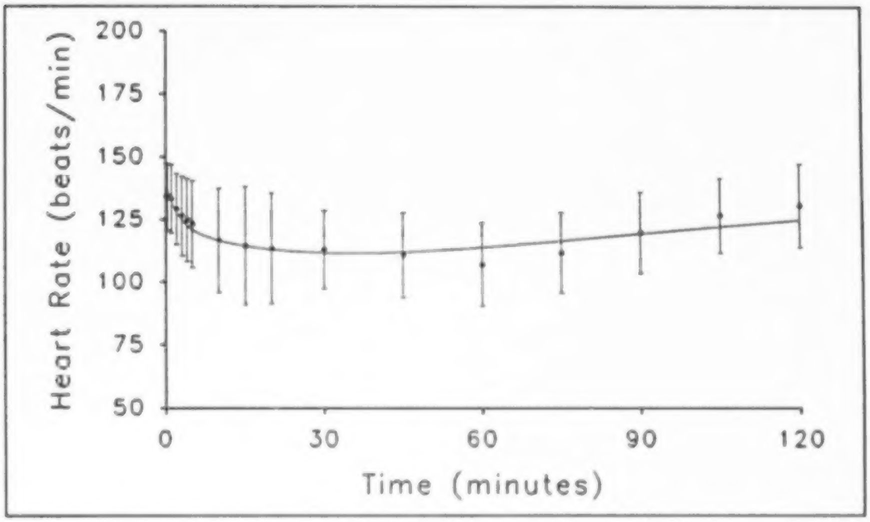

Figure 5.4 Heart rate versus time profile for captopril after a 0.2 mg/kg iv captopril dose to 5 piglets (means ± SD shown). Solid line represents model prediction.

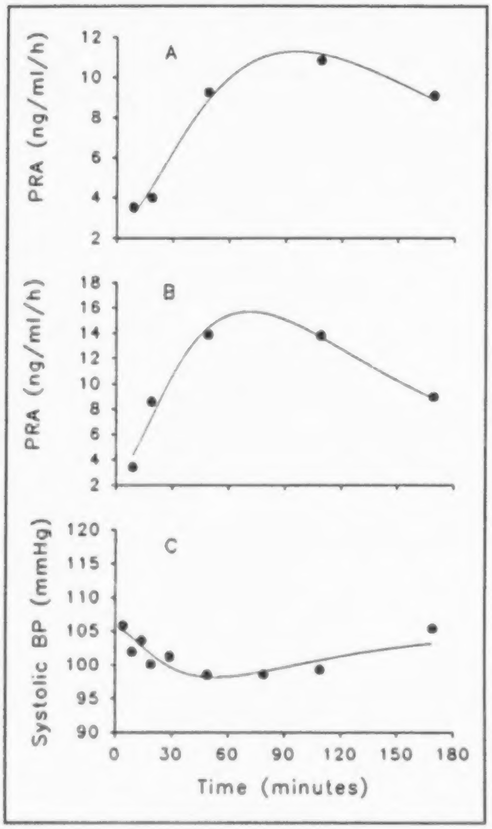

Figure 5.5 Plasma renin activity versus time profile after oral (A) and 'sublingual' (B) administration and systolic blood pressure (C) after 'sublingual' administration of 25 mg of captopril to healthy subjects (*Al-Furaih et al. 1991*). Solid lines represent model predictions.

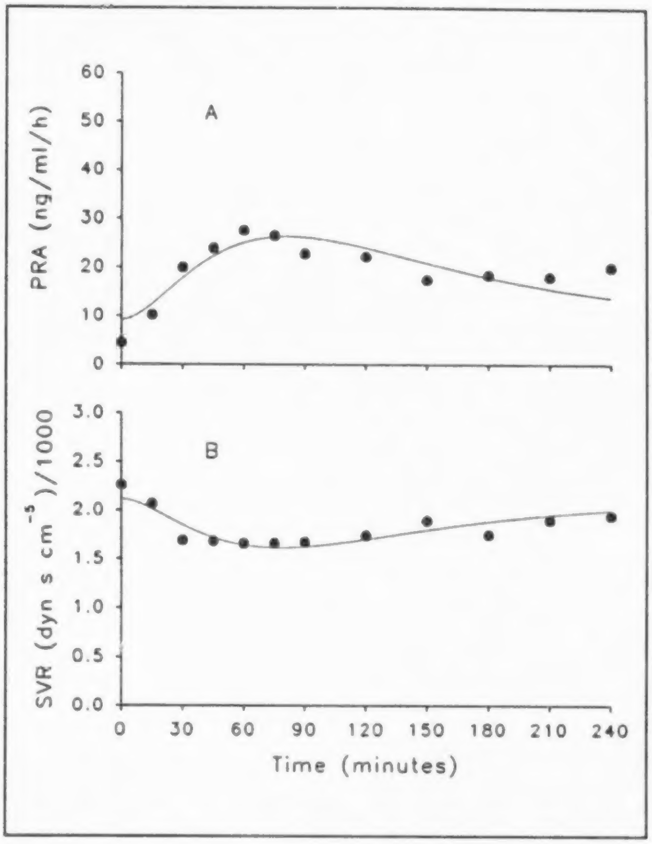

Figure 5.6 Plasma renin activity (A) and systemic vascular resistance (B) versus time profiles after administration of 25 mg captopril orally to patients with CHF (*Shaw et al. 1985*). Solid lines represent model predictions.

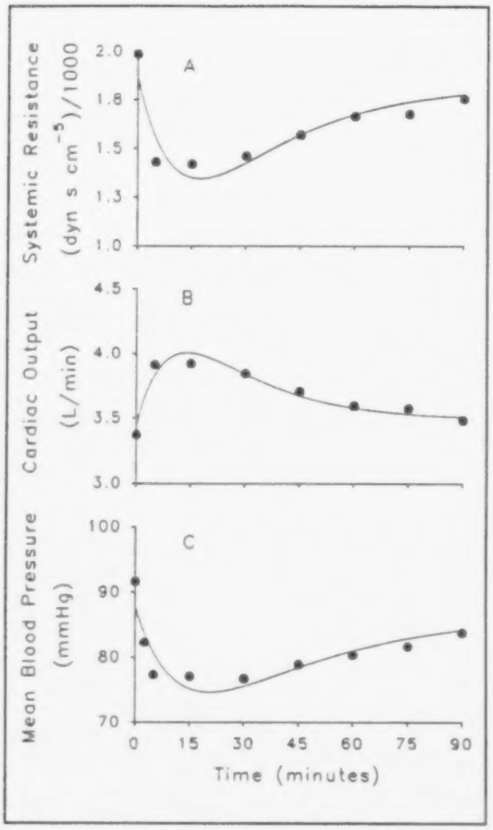

Figure 5.7 Systemic vascular resistance (A), cardiac output (B) and mean blood pressure versus time profiles after administration of 25 mg captopril intravenously to patients with congestive heart failure (*Rademaker et al. 1986*). Solid lines represent model predictions.

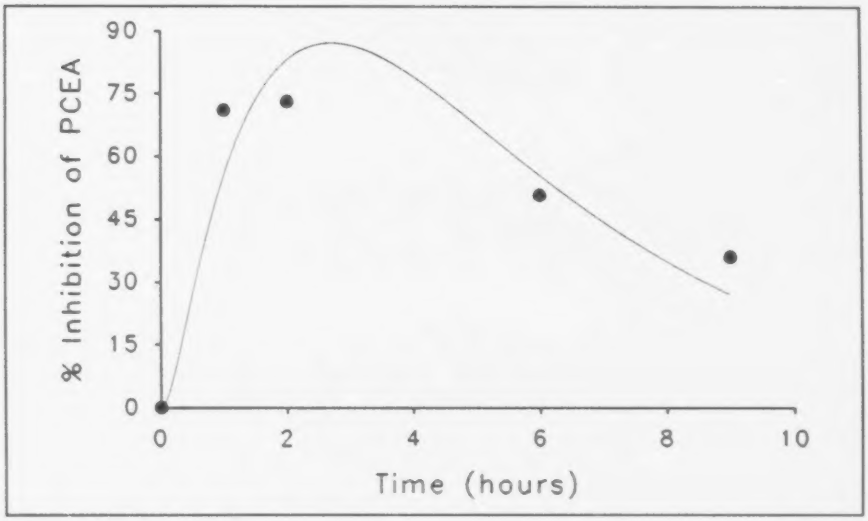

Figure 5.8 Percent inhibition of plasma converting enzyme activity versus time profile after administration of 50 mg captopril orally to hypertensive subjects (*Giudicelli et al. 1987*). Solid line represents model prediction.

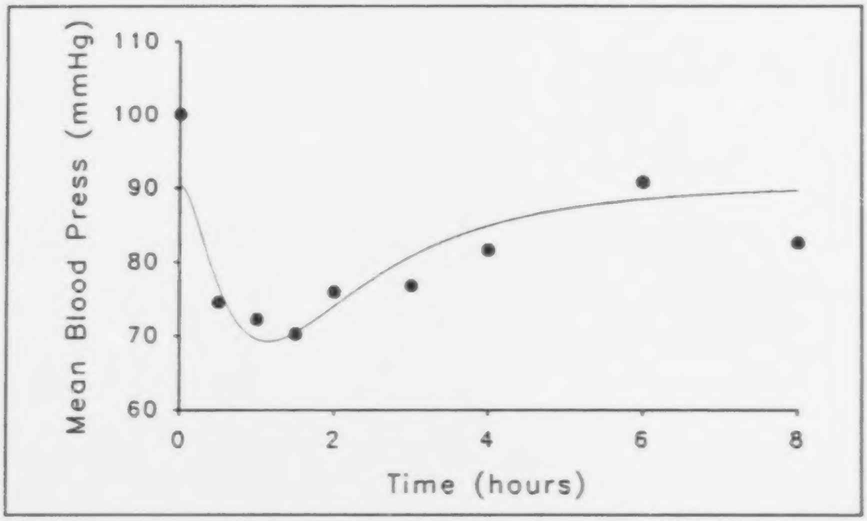

Figure 5.9 Mean blood pressure versus time profile after administration of 50 mg captopril orally to patients undergoing peritoneal dialysis (*Fujimura et al. 1986*). Solid line represents model prediction.

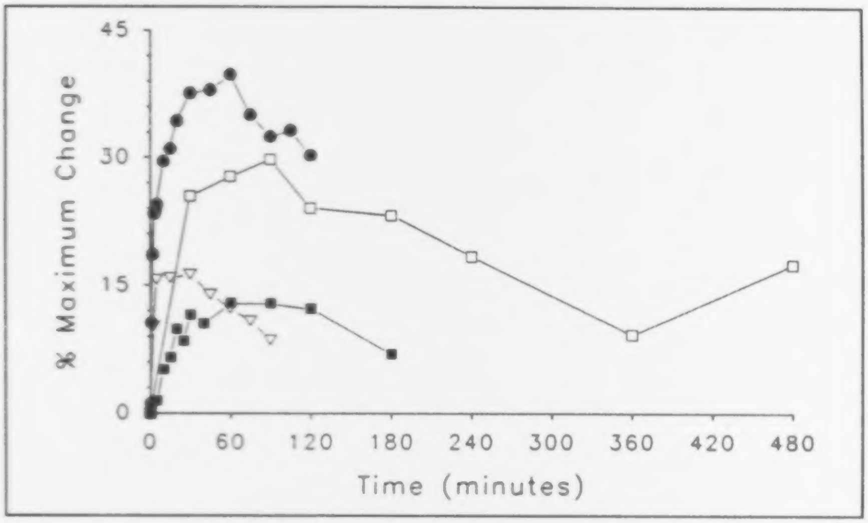

Figure 5.10 Maximum change percent versus time: (●) aortic pressure in piglets after captopril 0.2 mg/kg iv, (□) mean blood pressure in patients undergoing peritoneal dialysis, after 50 mg captopril orally (*Fujimura et al. 1986*), (▽) mean blood pressure in patients with CHF after 25 mg captopril iv (*Rademaker et al. 1986*), (■) systolic blood pressure in normal subjects after administration of 25 mg captopril orally (*Al-Furaih et al. 1991*).

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6. GENERAL DISCUSSION AND CONCLUSIONS

Captopril has been a very widely used drug since approval was given, in 1980, for its use in patients with hypertension (and subsequently for the treatment of congestive heart failure in 1984). Despite wide use there has, hitherto, been no pharmacokinetic data available for this drug in infants. In addition, prior to this investigation, no clear relationship between the pharmacokinetics and pharmacodynamics of captopril had been elucidated, even in adults. Several reports in the literature claimed that there was no relationship between plasma concentrations of captopril and the effects observed. The result has been that the use of captopril has been empirical. The first necessary step toward rectifying this situation would be to determine standard pharmacokinetic parameters in the infant population most likely to receive captopril i.e. infants with congestive heart failure. In order to achieve this objective we developed a sensitive and specific HPLC assay for the determination of captopril and its disulphides in plasma. Previously available assays for captopril were not suitable for use in studies involving infants because of the large volumes of blood the methods required. They were also cumbersome. Our relatively simple method requires a small volume of plasma and can therefore be used in paediatric studies.

There is presently no liquid dosage form of captopril available. The probable reason for this is that captopril has been shown to be unstable in various buffer solutions and biological fluids. Infants unable to swallow tablets are therefore given a solution of captopril (usually in tap water), freshly prepared for each dose. Apart from the economic consideration (important to some patients) of discarding a major portion of the drug solution, it was important to confirm that patients were receiving the proper dosage. We therefore studied the stability of captopril in tap water and concluded that the shelf-life of such solutions is approximately 26 days when they are stored at 5°C.

Having established an assay method, we were able to measure plasma captopril concentrations in infants and were therefore able to determine standard pharmacokinetic parameters for captopril in infants with congestive heart failure. The pharmacokinetic parameters for captopril in the infants were found to be within the range reported for adults with CHF. All infants were undergoing cardiac catheterization at the time of the first dose and changes in hemodynamic parameters observed at one hour post-dose suggested that captopril does indeed have a beneficial effect in these patients.

In order to model the relationship between plasma drug concentration and effect it is necessary to have serial concentration and relevant serial effect measurements. It was not feasible to obtain serial hemodynamic measurements in the infants and the pharmacodynamic data obtained were not sufficient to allow modelling of the concentration-effect relationship. An appropriate animal model was therefore deemed necessary. Swine have long been considered to be useful animal models in cardiovascular studies because of their anatomic and physiologic similarities to humans. The anaesthetized piglet was chosen in the hope that it would mimic the acute studies in sedated infants undergoing cardiac catheterization. The standard pharmacokinetic parameters for captopril determined in the piglet were found to be within the range reported for humans and the effects observed were qualitatively similar. Thus the anaesthetized piglet was considered to be a viable model in which to study the relationship between the kinetics and dynamics of captopril.

A parametric pharmacokinetic-pharmacodynamic model combined with a linear effect model was used to define a relationship between the kinetics and dynamics of captopril. The parametric kinetic-dynamic model included an effect compartment connected to the central compartment of a multi-compartment kinetic model. The model fit data obtained in the piglets as well as published data on humans very well.

We have therefore established a clear relationship between the pharmacokinetics and pharmacodynamics of captopril. The linear pharmacodynamic model appears to fit most clinical situations.